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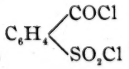
ON PARANITROORTHOTOLYLPHENYLSULPHONE AND SOME OF ITS DERIVATIVES.[1]

BY R. S. NORRIS.

INTRODUCTION.

In a series of investigations carried on in this laboratory on derivatives of orthosulphobenzoic acid, it has been shown that when its potassium salt is treated with phosphorus pentachloride two isomeric chlorides are formed,[2] the formulas of which appear to be :



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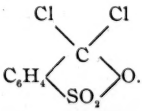


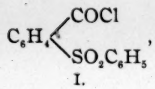
On treating either of these chlorides with aluminium chloride and benzene, one of the chlorine atoms is replaced by a phenyl group ; and the products formed are in both cases identical.[3] There must, therefore, be a rearrangement of the grouping of the atoms in one of the reactions.

There are three possible formulas for the product :

[1] From the author's dissertation submitted to the Board of University Studies of the Johns Hopkins University, June, 1897, for the degree of Doctor of Philosophy.

[2] Remsen and Dohme : This JOURNAL, **11**, 341 ; Remsen, et al. : *Ibid.*, **17**, 309 ; **18**, 791.

[3] Remsen and Saunders : *Ibid.*, **17**, 360.



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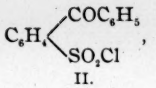

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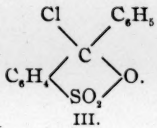
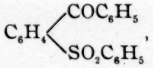
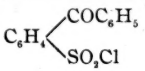


III.

From its properties and reactions its most probable structure seems to be that represented by the second formula, the phenyl residue being in combination with a carbonyl group. Remsen and Saunders[1] converted the chloride into the corresponding sulphonic acid by replacing the chlorine with a hydroxyl group, prepared a number of derivatives from this, and studied their properties. They also obtained a diphenyl derivative of orthosulphobenzoic acid of the formula,



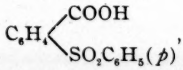
by the more prolonged action of benzene and aluminium chloride on the dichloride. The arguments which they bring forward in favor of the structure represented by the formula,



for the first product of the action are :

1. That the acid formed by the decomposition of the chloride with water is a very soluble substance, and difficult to obtain in a crystalline form.

This is characteristic of sulphonic acids in general, while, on the other hand, the corresponding carboxyl acids are much less soluble, and are easily obtainable in the form of crystals. Paraphenylsulphonebenzoic acid,



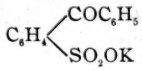
for instance, described by Newell,[2] is a difficultly soluble, crystalline compound. Another example will be given later in this paper.

[1] *Loc. cit.*

[2] This JOURNAL, **20**, 302.

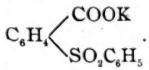
2. That the decomposition of the potassium salt by fusion with caustic potash gives benzoic acid and benzene sulphonic acid.

It is true that, on purely theoretical grounds, this might be a possible reaction with either of the substances represented by the formulas,



I.

and

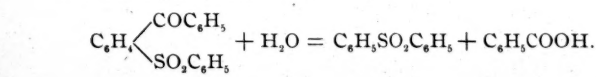


II.

But when we come to consider the experimental evidence on the point it seems much more probable that these products would result from a compound having the first formula than one having the second. Diphenylsulphone, for instance, when fused with caustic potash, breaks down completely into diphenyl, sulphur dioxide, phenol, and various other substances.[1] Tolylsulphone acts in a similar manner.[2] And these reactions take place with difficulty, showing the stability of the sulphone grouping,

$$C_6H_5.SO_2.C_6H_5.$$

The way in which the diphenyl substitution-product of orthosulphobenzoic acid breaks down also furnishes evidence in favor of this view. Remsen and Saunders found that it decomposes readily under similar circumstances into benzoic acid and diphenylsulphone,

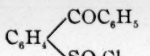


3. That the ethyl ether breaks down very readily into the acid on standing in contact with alcohol, which points to its being an ester of a sulphonic acid rather than of a carbonic acid.

4. That the chloride on treatment with ammonia, instead of forming an amide, yields a lactim. This is in accordance with the supposition that the chlorine atom left in the molecule is the one united with the sulphone group.

[1] Otto: Ber. d. chem. Ges., 19, 2425.
[2] Otto: *Loc. cit.*

The evidence, therefore, seems pretty clear that of the two formulas,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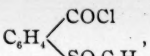
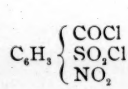


the former is the more probable for the compound obtained from the isomeric chlorides of orthosulphobenzoic acid by the Friedel and Crafts reaction with benzene.

Paranitroorthosulphobenzoic acid has also been found to yield two isomeric chlorides[1] by treatment with phosphorus pentachloride :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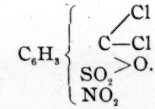
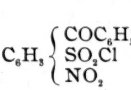
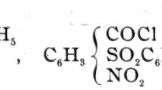
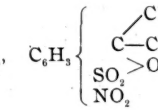


Both of these, in turn, when treated with benzene and aluminium chloride,[2] yield the same phenyl substitution-product having one of the three possible formulas :

I. II. III.

But the reaction seems in this case to stop here. No diphenyl substitution-product is formed by the further action of the reagents.

The same question arises in this case as in that of the compound just discussed : Which of the chlorine atoms is replaced by the phenyl group ? Hollis investigated the product formed and found from its properties and reactions somewhat similar evidence in favor of the first formula to that found by Remsen and Saunders in the former case. The acid obtained by decomposing the chloride with water was very soluble, and could not be obtained in the form of crystals. The ethyl ester of the acid, if one exists at all, is so unstable that it is imme-

[1] Remsen and Gray : This JOURNAL, **19**, 496.

[2] Hollis : *Ibid.*, **23**, 233.

diately transformed into the acid, when an attempt is made to prepare it from the chloride and absolute alcohol. On treating the chloride with ammonia a lactim is formed. These three reactions are perfectly analogous to those which take place with orthobenzoylbenzenesulphone chloride, under similar conditions, and point to the structure,

$$C_6H_3\begin{cases}COC_6H_5\\SO_2Cl\\NO_2\end{cases},$$

for the chloride. But the most conclusive evidence obtained by Remsen and Saunders for the formula which they gave, namely, the formation of certain products by fusing the acid with caustic potash, was not duplicated in the present case. The presence of the nitro group would probably make such a test less satisfactory. The evidence in this case, therefore, is not as strong as it is in that of the more simple compound.

There is, however, another method of attacking the problem by which just as conclusive evidence, but of a different kind, might be obtained. It was for the purpose of determining the nature of this evidence that the present investigation was undertaken.

If a compound having the formula, $C_6H_3\begin{cases}COCl\\SO_2C_6H_5\\NO_2\end{cases}$, could be prepared, and its properties should be different from the compound obtained by Hollis from the isomeric chlorides of paranitroorthosulphobenzoic acid, the latter could not have the same structure, and, therefore, would probably have the formula, $C_6H_3\begin{cases}COC_6H_5\\SO_2Cl\\NO_2\end{cases}$. If the properties should be the same, the structure of the two would, of course, be identical.

The best method that suggested itself for preparing paranitroorthophenylsulphonecarbonyl chloride was to start with paranitrotoluene, convert it into paranitroorthotoluenesulphonic acid by treatment with fuming sulphuric acid, treat the potassium salt of this with phosphorus pentachloride to form paranitroorthotoluenesulphone chloride from which paranitroorthotoluenephenylsulphone could be made by

the Friedel and Crafts reaction with benzene. By oxidation of the methyl group in this sulphone, paranitroorthophenylsulphonebenzoic acid would be formed, and this could be transformed into paranitroorthophenylsulphonebenzoyl chloride by the action of phosphorus pentachloride.

Another method which was suggested was to convert paranitrocyanbenzeneorthosulphonic acid, $C_6H_3(NO_2)\begin{cases}CN\\SO_3H(o)\end{cases}$, into its chloride, $C_6H_3(NO_2)\begin{cases}CN\\SO_2Cl\end{cases}$, replace the chlorine with phenyl, hydrolyze the cyan group into a carboxyl group, $C_6H_3(NO_2)\begin{cases}COOH\\SO_2C_6H_5\end{cases}$, and treat with phosphorus pentachloride to form paranitroorthophenylsulphonebenzoyl chloride,

$$C_6H_3(NO_2)\begin{cases}COCl\\SO_2C_6H_5\end{cases}.$$

Positive results were not obtained by this method.

Paranitroorthotoluenesulphone Chloride.

The paranitroorthotoluenesulphone chloride, prepared according to the directions of Jensen,[1] formed a yellow powder, melting at 43° to 45° C., readily soluble in ether, from which it crystallized in large, rhombic crystals, melting at 44° C. (uncorr.).

Although a number of different methods were tried, none was found to yield as good results as this one. Too high a temperature, or heating too long, decreases the yield, and makes the chloride dark. The purity of the product depends largely on that of the potassium salt used. The color of the latter was found to be carried over into the chloride.

Paranitroorthotoluenesulphone chloride has been described as a liquid.[2] The sharp melting-point of the product obtained by the above process, and crystallized from ether, is conclusive evidence of its purity; and the existence of the

[1] Ann. Chem. (Liebig), **172**, 230.
[2] Noyes: This JOURNAL, **8**, 167.

chloride in the liquid state at the ordinary temperature must be ascribed to the presence of some impurity. It is a common experience in working with solid sulphone chlorides, that the ease with which any particular specimen can be made to crystallize is much dependent on its purity.

Paranitroorthotolylphenylsulphone,

$$C_6H_3\begin{cases}CH_3\\SO_2C_6H_5(o).\\NO_2(p)\end{cases}$$

This was obtained from the chloride just described, by means of the Friedel and Crafts reaction with benzene.

Limpricht[1] obtained a compound isomeric with this, by the action of aluminium chloride and toluene on metanitrobenzenesulphone chloride,

$$C_6H_4\begin{cases}NO_2(m)\\SO_2Cl\end{cases} + H.C_6H_4CH_3 \rightarrow C_6H_4\begin{cases}NO_2\\SO_2.C_6H_4.CH_3\end{cases},$$

the nitro and methyl groups being attached to different benzene rings. From the properties which are given, it seems to be quite different from paranitroorthotolylphenylsulphone, except in solubility. One of these points of difference will be taken up later under the head of the action of oxidizing agents on the sulphone.

Preparation and Properties.

As a preliminary experiment, 10 grams of the chloride were dissolved in about 100 cc. of benzene, in a small flask provided with a condensing tube. About 4 grams of aluminium chloride, in small pieces, were added, and the solution warmed gently. Action began slowly with the evolution of hydrochloric acid from the surface of the aluminium chloride. The solution began to darken quickly, and, after heating for five minutes, the action was stopped by cooling the flask, and the contents were poured into dilute hydrochloric acid. The benzene solution was then separated from the water and evaporated to dryness. The residue—a dark-brown, tarry mass—was treated in different portions with alcohol, ether, chloro-

[1] Ann. Chem. (Liebig), **278**, 257.

form, and benzene, and the solutions filtered. Crystals separated from each of the solutions on evaporation, but the ether and alcohol dissolved less of the dark tarry substance, and consequently furnished the best crystals.

It was found, on further experimenting, that the aluminium chloride reacted more satisfactorily when powdered, and that it was not necessary to use such a large proportion of benzene. The darkening, which takes place rapidly after the action begins, could not be prevented, and it was found to have less effect on the final product than was at first supposed.

Previously to using the method finally adopted the yield was small, only 6 grams of the sulphone being obtained from 35 grams of the chloride. This method is similar to that used by Beckurts and Otto[1] in preparing paratolylphenylsulphone from benzenesulphone chloride and toluene, and by Newell[2] in making the same substance from paratoluenesulphone chloride and benzene. Only 20 grams of the chloride were used at a time, as this was found to give the best results. It was dissolved in an equal weight of warm benzene in a flask connected with a condensing tube. The solution was placed on a water-bath and heated so that the benzene solution boiled gently. Fifteen grams of powdered aluminium chloride were added gradually, 3 or 4 grams at a time—the violent action being allowed to subside after each addition. The solution was heated for half an hour, allowed to cool, poured into a liter of water containing 20 to 30 cc. of concentrated hydrochloric acid. A voluminous light-brown, flocculent precipitate formed. The solution was warmed on the water-bath until all of the benzene had evaporated, the precipitate filtered off, and drained with the filter-pump. This process was repeated until 200 grams of the chloride had been used. Most of the precipitate was found to be soluble in alcohol, but the yield was considerably decreased in the process of purification.

The collected residues were extracted with hot alcohol, and the alcoholic solution boiled with animal charcoal until nearly colorless, and filtered while hot with the use of a hot-water funnel. The marked difference in the solubility of the sul-

[1] Ber. d. chem. Ges., 11, 2068.
[2] *Loc. cit.*

phone in hot and cold alcohol caused considerable difficulty in its purification. Most of it crystallized out from the alcohol on cooling, in the form of thin, silver-white plates, melting at 158° C. (uncorr.). That which remained dissolved in the cold alcohol was obtained by concentrating the solution. This part of the product was not as pure as that which separated out from the original solution, as it was found that an alcoholic solution of the sulphone became brown when boiled for a while, or even allowed to stand at the ordinary temperature for more than a day. The charcoal took up a considerable amount of the sulphone, which was recovered by extracting with alcohol.

Analysis of the product gave the following results:

I. 0.3140 gram substance gave 0.2652 gram $BaSO_4$.

II. 0.1583 gram substance gave 0.1356 gram $BaSO_4$.

III. 0.2863 gram substance gave 0.5934 gram CO_2, and 0.1058 gram H_2O.

IV. 0.2994 gram substance gave 0.6201 gram CO_2, and 0.1063 gram H_2O.

V. 0.3229 gram substance gave 0.017 gram N.

	Calculated for $C_{13}H_{11}O_4NS$.	Found.				
		I.	II.	III.	IV.	V.
C	56.30	....	....	56.56	56.52	....
H	4.00	....	....	4.13	3.93	....
S	11.56	11.60	11.76	....	....	....
N	5.06	....	....	....	....	5.26

Paranitroorthotolylphenylsulphone exhibits, in a marked degree, the general stability of sulphones toward concentrated acids. It is, however, much less stable toward alkalies than the simpler sulphones, undoubtedly on account of the presence of the nitro group. Most of the ordinary organic solvents dissolve it easily.

It was found to be nearly as soluble in methyl alcohol as in ethyl alcohol. Benzene and acetone dissolve it very readily, and it crystallizes out from both in plates, which, in the latter case, are very narrow, and to the naked eye have the appearance of needles. It is almost equally soluble in hot and cold ether, and to rather a small extent. Hot ligroin dissolves it with ease. In water it is practically insoluble, even in boil-

ing water; but that it does dissolve to a limited extent is shown by the fact that the filtrate from a water solution gives a faint yellow color when boiled with a solution of caustic potash. This is a very delicate test, as one small crystal of the sulphone, dissolved in alcohol, gives a distinct red color with such a solution.

Dilute nitric and sulphuric acids dissolve the sulphone in small amounts; dilute hydrochloric acid does not dissolve it. Concentrated nitric and sulphuric acids dissolve it quite appreciably in the cold, and give it up again on dilution with water. A small amount was boiled with concentrated nitric acid for five minutes, the solution cooled, and diluted with water. The sulphone was precipitated in crystalline form, and it melted sharply at 158° C., showing that it had not been acted upon by the acid. A similar experiment was tried with fuming nitric acid, with the same result. A mixture of equal parts by volume of concentrated nitric and sulphuric acids was then tried; the sulphone which crystallized out on dilution had a sharp melting-point. When boiled with a mixture of concentrated sulphuric and fuming nitric acids it was slightly decomposed. There was no apparent action, but the white precipitate which formed on dilution did not melt sharply.

The sulphone dissolved quite readily in ordinary concentrated sulphuric acid in the cold, and was precipitated unchanged when the solution was poured into water. When the acid was warmed to 100° C. the solubility of the sulphone increased, but it was not acted upon. When the temperature was raised much above this the sulphone was decomposed, as shown by the charring. Fuming sulphuric acid dissolved the sulphone easily, and it was not precipitated on dilution.

Action of Fuming Sulphuric Acid.

When the sulphone is treated with fuming sulphuric acid it is converted into a sulphonic acid. An analysis of the barium salt of this acid is here given:

0.0849 gram substance lost 0.0097 gram at 150° C., and gave 0.0209 gram $BaSO_4$.

	Calculated for $(C_{13}H_{10}O_7S_2N)_2Ba + 6H_2O$.	Calculated for $(C_{13}H_{10}O_7S_2N)_2Ba$.	Found.
H_2O	11.28	....	11.42
Ba	....	16.16	16.35

Action of Potassium Hydroxide.

A mere trace of the sulphone in solution gives a purplish-red color with caustic potash.

A few crystals of the sulphone were dissolved in alcohol and the solution of caustic potash added. Upon being heated the solution quickly assumed a deep purple color, which became red after boiling for a while, and finally a purplish-red precipitate formed. Dilute hydrochloric, nitric, and sulphuric acids were added to different portions of the solution. They changed the color to reddish-yellow, and the purple color was restored on addition of excess of potassium hydroxide. The purple color was not brought back, however, with either ammonium hydrate or sodium carbonate. Concentrated sulphuric, nitric, and hydrochloric acids each produced apparently the same result as the dilute acids. But when the solutions were made alkaline again with caustic potash, the purple color did not return. The purple-colored compound was evidently decomposed by the concentrated acids.

Caustic soda had the same effect on the sulphone as caustic potash. Ammonium hydrate and sodium carbonate had no action on the sulphone.

An attempt was made to isolate the compound formed. About 5 grams of the sulphone were dissolved in absolute alcohol, and treated with an alcoholic solution of pure potassium hydroxide. The solution was boiled for five minutes, cooled, and poured into about a liter of water. A red flocculent precipitate settled to the bottom of the vessel, leaving the supernatant liquid clear and not very deeply colored. The precipitate was washed by decantation until no longer alkaline, then boiled with water and washed again. After filtering off and drying, its solubility in a number of solvents was tried for the purpose of finding a method for purifying it. Treated with alcohol, ether, acetone, and acetic acid, it gave a slight red color to each, but did not dissolve appreciably. Evaporation of the solutions in each case left but a trace of amorphous residue. Concentrated sulphuric acid dissolves it to a certain extent, forming a deep purple-colored solution. On diluting, the color changed to reddish-yellow, and the

original purple color did not appear again, on making the solution alkaline with potassium hydroxide. The substance left quite a large amount of ash when ignited, consisting evidently of the potassium hydroxide that could not be removed by washing. On account of its insolubility the substance could not be obtained pure.

Action of Reducing Agents :—Ammonium Sulphide.

Gericke[1] found that when nitrophenylsulphone in alcoholic solution is treated with ammonium sulphide and heated it is reduced to amidophenylsulphone. Noyes[2] has shown also that paranitroorthosulphobenzoic acid is easily reduced by the same reagent to paramidoorthosulphobenzoic acid.

The nitro group of paranitroorthotolylphenylsulphone was also found to be readily converted into an amido group in the same way.

Paramidoorthotolylphenylsulphone, $C_6H_3(NH_2)\begin{cases}CH_3\\SO_2C_6H_5\end{cases}$. — Four grams of paranitroorthotolylphenylsulphone were dissolved in 100 cc. of hot alcohol, about 20 cc. of concentrated ammonia water added, and sulphuretted hydrogen passed into the solution for fifteen minutes. The solution became blood-red in a short time. A small amount of the solution was removed and treated with water, which caused a voluminous precipitate of short, needle-shaped crystals. The main portion of the solution was then evaporated to dryness on a water-bath, and the residue digested with water containing a small amount of hydrochloric acid. The portion remaining undissolved, which consisted of sulphur, was removed by filtration through a layer of flowers of sulphur in a Gooch crucible. On making the filtrate alkaline with caustic soda, a heavy white precipitate came down. This was filtered off, washed, and drained with the filter-pump. It was then dissolved in alcohol, treated with animal charcoal, boiled, filtered, and allowed to crystallize out. The crystals were large, thin, irregular-shaped, colorless plates, and melted at 156° C. (uncorr.). They were difficultly soluble in hot water, and crys-

[1] Ann. Chem. (Liebig), **100**, 208.
[2] This JOURNAL, **8**, 167.

tallized out from this solution in very small plates. Alcohol and acetone dissolved them readily, and they were not precipitated from either solution on addition of water. They dissolved to only a small extent in either cold or boiling ether. Benzene dissolved them very readily, and the compound crystallized out in the form of needles.

I. 0.1681 gram substance gave 8 cc. N at 19° C., and 765 mm. Hg pressure = 0.009264 gram.

II. 0.3496 gram substance gave 17.5 cc. N at 20° C., and 755 mm. Hg pressure = 0.0199 gram.

	Calculated for $C_{13}H_{13}O_2NS$.	Found. I.	Found. II.
N	5.67	5.51	5.69

Action of Oxidizing Agents.

1. *Chromic Acid.*—Newell[1] found that the best method for converting paratolylphenylsulphone into paraphenylsulphonebenzoic acid, is by treatment with chromic acid in glacial acetic acid solution. The same method was tried with this sulphone.

Ten grams of the sulphone were dissolved in glacial acetic acid and heated to boiling in a flask provided with a return condenser. A saturated solution of about 12 grams of chromic acid in glacial acetic acid was added, in small quantities at a time, the solution being kept just hot enough to boil gently. After all the chromic acid had been added, the solution was boiled for three hours. It was filtered and the acetic acid evaporated from the filtrate. The original precipitate was found to consist of chromic oxide, almost free from organic matter. The residue from the filtrate consisted principally of unaltered sulphone mixed with the chromic acid that remained.

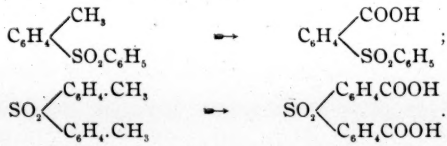
The action of chromic acid in this case, or rather its lack of action, is in accord with its usual behavior toward ortho substitution-products of toluene. The presence of the phenylsulphone group, $SO_2C_6H_5$, in the ortho position to the methyl group, seems to shield the latter from the action of chromic acid.

2. *Potassium Permanganate.*—The most efficient oxidizing agent for the ortho-substituted toluenes has generally been

[1] *Loc. cit.*

found to be potassium permanganate in water solution.

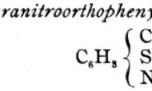
The action of oxidizing agents on sulphones was first investigated by R. Otto. He found[1] that when diphenyl sulphone, mixed with water acidified with sulphuric acid, is treated with potassium permanganate, the permanganate is somewhat reduced, as shown by the precipitation of manganese dioxide. Michael and Adair[2] showed later that tolylphenylsulphone and ditolylsulphone are oxidized by potassium permanganate to a phenylsulphonebenzoic acid and a phenylsulphonedibenzoic acid, respectively :



When the reaction was carried on in acetic acid solution the yield was small. A water solution of potassium permanganate gave much better results, although the sulphone was but little soluble in water, and only half of it was oxidized by the theoretical quantity of potassium permanganate.

Paranitroorthotolylphenylsulphone was found to act in a similar manner.

Paranitroorthophenylsulphonebenzoic Acid,



The acid was prepared by the following method :

Ten grams of the sulphone were suspended in a liter of water in a 1.5 liter flask. The sulphone had previously been powdered as fine as possible in a mortar. A solution of 30 grams of potassium permanganate was added, one-quarter at a time, at intervals of an hour, to the solution which was heated to boiling meanwhile on a sand-bath. The color of the permanganate was gradually displaced by that of the manganese dioxide as the reaction progressed. It was difficult to determine, from the appearance of the solution, when the last

[1] Ann. Chem. (Liebig), **145**, 37.
[2] Ber. d. chem. Ges., **11**, 119.

traces of the permanganate had disappeared, without letting the solution settle, which required considerable time. A small amount of the solution was removed occasionally and filtered, and when a colorless filtrate was obtained the heating was stopped. It usually required eight hours to decompose all of the potassium permanganate. The solution was allowed to cool, and filtered. On acidifying the filtrate with hydrochloric acid a finely divided, white precipitate came down, and, on standing, it collected in masses of irregular, needle-shaped crystals. It was filtered off and purified by redissolving it in water, and treating with animal charcoal. The largest yield obtained at one time was 2.8 grams of the acid from 10 grams of the sulphone. A considerable amount of sulphone was always left unacted upon, and mixed with the precipitate of manganese dioxide. It was recovered by dissolving the dioxide in concentrated hydrochloric acid and filtering off the undissolved sulphone, which was not attacked by the nascent chlorine.

Paranitroorthophenylsulphonebenzoic acid crystallizes in white needles 5 to 10 mm. long, melting at 196° C. (uncorr.). It is difficultly soluble in cold water, and not very readily in hot water. It has a bitter-sweet taste. Acetone dissolves it readily, but ether and benzene only to a small extent.

Barium nitrophenylsulphonebenzoate, $(C_6H_3NO_2SO_2C_6H_5.COO)_2Ba.H_2O$, crystallizes in the form of short, white needles, matted together, and difficultly soluble in hot water. Analysis gave the following results:

I. 0.3784 gram substance last 0.0100 gram at 178° C., and gave 0.1146 gram $BaSO_4$.

II. 0.5043 gram substance lost 0.0125 gram at 178° C., and gave 0.1524 gram $BaSO_4$.

	Calculated for $(C_{13}H_9O_6NS)_2Ba.H_2O$.	Calculated for $(C_{13}H_8O_6NS)_2Ba$.	Found. I.	Found. II.
H_2O	2.34	....	2.64	2.48
Ba	....	18.32	18.30	18.23

Calcium nitrophenylsulphonebenzoate, $(C_6H_3NO_2C_6H_5COO)_2Ca.6\frac{1}{2}H_2O$, crystallizes in tufts of radiating, colorless, prismatic, crystals, easily soluble in hot water. Analysis of the salt gave the following results:

0.1907 gram substance lost 0.0292 gram at 160° C., and gave 0.0342 gram $CaSO_4$.

	Calculated for $(C_{13}H_8O_6NS)_2Ca.H_2O$.	Calculated for $(C_{13}H_8O_6NS)_2Ca$.	Found.
H_2O	15.21	....	15.31
Ca	....	6.14	6.23

Comparison of Paranitroorthophenylsulphonebenzoic Acid with Paranitroorthobenzoylbenzenesulphonic Acid.

Hollis[1] found that the acid formed by the action of water and dilute acids on the product of the Friedel and Crafts reaction with the chlorides of paranitroorthosulphobenzoic acid, is very easily soluble in water, and cannot be obtained in crystallized form. Its barium salt crystallizes with varying amounts of water of crystallization, but never with less than 3 molecules. The crystals have the form of tufts of radiating needles or monoclinic prisms. Its calcium salt crystallizes in thin plates with 3 molecules of water of crystallization.

Paranitroorthophenylsulphonebenzoic acid, on the other hand, is difficultly soluble in water and crystallizes easily. Its barium salt crystallizes as short needles, matted together, and containing 1 molecule of water of crystallization; and its calcium salt crystallizes in prisms containing 6.5 molecules of water of crystallization.

The difference can be brought out more strikingly by means of the following table:

Paranitroorthobenzoylbenzenesulphonic Acid.	*Paranitroorthophenylsulphonebenzoic Acid.*
Very soluble in water.	Difficultly soluble in water.
Not crystallizable.	Crystallizes easily.
Barium salt contains from 3 to 7 molecules of water of crystallization, and crystallizes in tufts of radiating needles or monoclinic prisms.	Barium salt contains 1 molecule of water of crystallization, and crystallizes in short, matted needles.
Calcium salt crystallizes in plates with 3 molecules of water of crystallization.	Calcium salt crystallizes in prisms with 6.5 molecules of water of crystallization.

[1] *Loc. cit.*

Action of Phosphorus Pentachloride on Paranitroorthophenylsulphonebenzoic Acid.

Phosphorus pentachloride acts readily on the free acid, to form paranitroorthophenylsulphonebenzoyl chloride.

When the acid was intimately mixed with phosphorus pentachloride, free from oxychloride, no action was noticed until the temperature was raised to 160° C. But when the pentachloride contained phosphorus oxychloride, the reaction began immediately at the ordinary temperature on mixing the two together. The reaction which takes place may be represented thus :

$$C_6H_2\begin{cases}COOH\\SO_2C_6H_5\\NO_2\end{cases} + PCl_5 = C_6H_3\begin{cases}COCl\\SO_2C_6H_5\\NO_2\end{cases} + HCl + POCl_3.$$

Paranitroorthophenylsulphonebenzoyl Chloride.

Equal weights of paranitroorthophenylsulphonebenzoic acid and phosphorus pentachloride containing a small amount of the oxychloride were intimately mixed in a mortar. The mixture gradually softened into a semiliquid mass, with rise of temperature, and liberation of hydrochloric acid and phosphorus oxychloride. After the action was over the mass was transferred to an evaporating dish and heated about ten minutes longer at about 150° C. It then had the appearance of a light-brown, transparent liquid. It was allowed to cool to the ordinary temperature, and washed with cold water, which caused it to solidify to a light-yellow, granular solid. It was reduced to a fine powder and washed until free from phosphoric acid, dried with drying-paper, and dissolved in warm chloroform. It crystallized out of the solution on the spontaneous evaporation of the chloroform, as well-formed, colorless, rhombic crystals, from 1 to 5 mm. long, melting at 109° C. (uncorr.). The crystals were easily soluble in chloroform, ether, benzene, and ligroin.

The chlorine in the chloride was determined by boiling with a dilute solution of pure caustic soda (made from metallic sodium), acidifying with nitric acid, and precipitating with silver nitrate.

I. 0.1631 gram substance gave 0.0716 gram AgCl.
II. 0.1847 gram substance gave 0.0810 gram AgCl.

	Calculated for $C_{13}H_9O_5NSCl$.	Found. I.	II.
Cl	10.88	10.85	10.84

Action of Water.

The chloride is quite stable in the presence of water at ordinary temperatures. No change could be detected in crystals which remained in contact with water several days. But it is readily decomposed when boiled with water.

Dilute caustic alkalies in solution decompose the chloride much more quickly than water. Two-tenths gram of the chloride was entirely decomposed when boiled with a very dilute solution of caustic potash for five minutes. Hydrochloric acid was added in excess to the solution, and very fine, needle-shaped crystals separated out on cooling. Their melting-point, 196° C., showed them to be paranitroorthophenylsulphonebenzoic acid.

Action of Ammonia.

By the action of ammonia the chlorine of the carbonyl chloride group is replaced by an amido residue.

Some of the finely powdered chloride was added to a dilute solution of ammonia in a flask and allowed to remain in contact with it for several days. The solid material in the bottom of the flask at the end of this time had the same appearance as the chloride—a light-yellow powder. The ammonia was evaporated off on a water-bath, and the solution filtered from the residue, which was washed, dried, and dissolved in hot chloroform, in which it was not very readily soluble. When the chloroform evaporated, it crystallized out in well-formed, colorless, prismatic crystals, apparently monoclinic, and melting at 191° to 192° C. (uncorr.). The crystals were analyzed with the following results:

I. 0.4049 gram substance gave 0.3004 gram $BaSO_4$.
II. 0.1515 gram substance gave 0.01385 gram N.

	Calculated for $C_{13}H_{10}O_5N_2S$.	Found. I.	II.
S	10.46	10.19	....
N	9.16	....	9.14

The product is

Paranitroorthophenylsulphonebenzamide.—The amide is practically insoluble in water, benzene, and ether. It dissolves readily in hot alcohol, and crystallizes out as the solution cools, in the form of prisms. Acetone also dissolves it very easily, and well-formed prisms crystallize out on evaporation of the acetone.

Action of Benzene and Aluminium Chloride.

On adding aluminium chloride to a warm solution of the orthosulphonebenzoyl chloride in benzene, a slight evolution of hydrochloric acid took place, and a brown, tarry mass, which was insoluble in the benzene, very soon collected around the aluminium chloride and prevented further action.

Two grams of the chloride were dissolved in about 10 grams of benzene, and 2 grams of powdered aluminium chloride added. The solution was heated on a water-bath until it just began to boil, and it was then kept at this temperature. Hydrochloric acid was given off quite rapidly at first, passing out in a continuous stream from the condensing-tube. After the evolution of acid had ceased, the solution was cooled and poured into water. The mixture was heated on the water-bath until the benzene had evaporated. The tarry mass which remained at the bottom of the beaker was found to be insoluble in benzene, alcohol, ether, chloroform, and acids. It did not, therefore, lend itself to further investigation.

Lack of material prevented the carrying out of any more experiments of this kind.

Comparison of Paranitroorthophenylsulphonebenzoyl Chloride with Paranitroorthobenzoylbenzenesulphone Chloride.

The compound obtained by Hollis[1] from the action of benzene and aluminium chloride on the isomeric chlorides of paranitroorthosulphobenzoic acid, crystallizes from its solution in benzene in the form of purple or green monoclinic crystals, melting at 177° C. It yields a non-crystallizable acid when boiled with water, and when treated with ammonia it combines with it, with the loss of hydrochloric acid and water, to

[1] *Loc. cit.*

form a lactim which melts at 234° C., and which is not very soluble in alcohol. Its reactions, therefore, are in accordance with what we should expect from a compound having the formula,

$$C_6H_3\begin{cases}COC_6H_5\\SO_2Cl\\NO_2\end{cases}.$$

Paranitroorthophenylsulphonebenzoyl chloride, isomeric with the above compound, crystallizes from benzene in colorless or light-yellow, rhombic crystals, melting at 109° C. When boiled with water, it forms a well-crystallized acid, which melts at 196° C.; and it reacts with ammonia to form an amide crystallizing in prisms which melt at 191° to 192° C., and are readily soluble in alcohol. These properties characterize it as quite distinct from the compound of the same empirical composition described above.

An Attempt to Transform Paranitroorthocyanbenzenesulphonic Acid into Paranitroorthophenylsulphonebenzoic Acid.

The unsymmetrical chloride of paranitroorthosulphobenzoic acid is converted into the ammonium salt of paranitroorthocyanbenzenesulphonic acid, $C_6H_3\begin{cases}CN\\SO_2ONH_4\\NO_2\end{cases}$, when treated with a solution of ammonia. It was thought that this substance might furnish the starting-point for another method for the preparation of paranitroorthophenylsulphonebenzoic acid. By the action of phosphorus pentachloride on the potassium salt of this acid, paranitroorthocyanbenzenesulphone chloride, $C_6H_3\begin{cases}CN\\SO_2Cl\\NO_2\end{cases}$, is formed.[1] It was hoped that by the action of benzene and aluminium chloride this would yield paranitroorthocyandiphenylsulphone according to the reaction,

$$C_6H_3\begin{cases}CN\\SO_2Cl\\NO_2\end{cases} + HC_6H_5 \longrightarrow C_6H_3\begin{cases}CN\\SO_2C_6H_5\\NO_2\end{cases},$$

which, by hydrolysis of the cyan residue, would give paranitroorthophenylsulphonebenzoic acid,

[1] Remsen and Gray: This JOURNAL, 19, 510.

$$C_6H_3\begin{cases}CN\\SO_2C_6H_5\\NO_2\end{cases} + 2H_2O \rightarrow C_6H_3\begin{cases}COOH\\SO_2C_6H_5.\\NO_2\end{cases}$$

The material for the experiments was prepared by Mr. W. E. Henderson, of this laboratory. The ammonium salt, which was obtained as a waste-product in the preparation of the symmetrical chloride of paranitroorthosulphobenzoic acid, was converted into the potassium salt by adding caustic potash or potassium chloride to its solution in hot water. The potassium salt is difficultly soluble in cold water, and separates out in the form of needles as the solution cools. Potassium chloride was found to work more satisfactorily than potassium hydrate. An analysis by Mr. Henderson of the potassium salt prepared in this way confirmed its purity. The salt was heated two or three hours in an air-bath at about 130° C. to free it from water of crystallization.

Twenty-five grams of the dry potassium salt were mixed in a mortar with an equal weight of phosphorus pentachloride, placed in an evaporating dish, and heated to 140° C. on a sulphuric-acid bath. After the reaction had ceased, the heating was continued half an hour longer to evaporate off the phosphorus oxychloride. It was allowed to cool to the ordinary temperature and treated with cold water, which caused it to solidify. It was ground to a fine powder, washed several times with water, drained, and dissolved in chloroform. A small portion remained undissolved, which was probably the anhydride of the acid. Some of the acid was also recovered from the water used in washing the chloride, showing that the use of a larger quantity of phosphorus pentachloride is necessary to convert all of the potassium salt into the chloride. The chloroform solution was freed from water by calcium chloride, and allowed to evaporate spontaneously. Large, well-formed, quadrilateral, prismatic crystals were obtained, which melted at 104°.5 to 105°.5 C. (uncorr.).

Ten grams of the chloride were dissolved in an equal weight of warm benzene, in a flask provided with a condensing-tube, and 8 grams of powdered aluminium chloride added gradually, in small portions at a time. No reaction took place until the solution was warmed. A brown, tarry mass then collected at

the bottom of the flask, forming a solid cake with the aluminium chloride. The solution was heated on the water-bath until hydrochloric acid was no longer given off. Although considerable hydrochloric acid was liberated, there was no apparent evolution from the surface of the aluminium chloride, as is usually the case in the Friedel and Crafts reaction. The benzene solution, which had a slight-brown color, was poured into water, and the mixture heated on the water-bath until the benzene had all evaporated. Nothing remained in the water but a small amount of undecomposed chloride. The residue in the flask was washed with dilute hydrochloric acid and extracted with alcohol. That which remained undissolved in the alcohol was a brown, amorphous powder, insoluble in ether, benzene, chloroform, and concentrated acids. The alcoholic extract was evaporated to dryness on the water-bath, leaving a brown, tarry mass, from which no crystalline product could be obtained. It was dissolved in ether, chloroform, benzene, and acetone, but separated out in the same form on evaporation of the solutions.

The experiment was repeated with 100 cc. of benzene and 10 grams of the chloride, and the result was essentially the same.

The presence of the cyan group evidently interferes with the action of benzene on the sulphone chloride residue.

Conclusions.

The results of this investigation lead to the following conclusions:

1. Paranitroorthotolylphenylsulphone can be readily obtained by the action of benzene and aluminium chloride on paranitroorthotoluenesulphone chloride.

2. This sulphone has the general properties of aromatic sulphones, and certain other properties due to the presence of the nitro group.

3. Paranitroorthotolylphenylsulphone is oxidized by potassium permanganate in neutral solution to paranitroorthophenylsulphonebenzoic acid, and this acid is quite different in properties from the isomeric compound obtained by decomposing with water, the product of the action of benzene and

aluminium chloride on the chlorides of paranitroorthosulphobenzoic acid.

4. Paranitroorthophenylsulphonebenzoic acid passes over quantitatively into paranitroorthophenylsulphonebenzoyl chloride when treated with phosphorus pentachloride.

5. Paranitroorthophenylsulphonebenzoyl chloride has perfectly definite properties, which distinguish it from the isomeric compound obtained by the action of benzene and aluminium chloride on the chlorides of paranitroorthosulphobenzoic acid. Among other differences, attention is called to the fact that it forms an amide with ammonia, while the latter forms a lactim.

CONCERNING LIPASE, THE FAT-SPLITTING ENZYME, AND THE REVERSIBILITY OF ITS ACTION.

By J. H. Kastle and A. S. Loevenhart.

It was suggested by one of us (Loevenhart) that possibly lipase might be capable of effecting the synthesis of fats from fatty acid and glycerin as well as their hydrolysis into these products. It was with the view of making an experimental study of this question that this investigation was first undertaken. An examination of the literature of the subject revealed that but comparatively little was known concerning the fat-splitting enzyme. It was therefore deemed advisable to extend the original scope of the investigation. The most convenient source of the enzyme to us was the pancreas of the pig. In the preparation of our extracts, the organ was removed from the animal within thirty minutes after death, dissected as free as possible from fat, thoroughly macerated with coarse white sand, and extracted with water or glycerin. It was afterwards found more convenient, however, to cut up the pancreas in small pieces upon its removal from the animal, and preserve it in chloroform water in cold storage until required for use. At first the activity of the enzyme was tested upon pure butter-fat, litmus being used as the indicator and the time being noted that was required

to pass from the blue color to a definite shade of red. Under the best conditions, however, lipase hydrolyzes the fats but slowly, and hence they are by no means convenient substances with which to follow the change in question. In testing the activity of the pancreas extract on other ethereal salts, it was observed that it hydrolyzed ethyl butyrate comparatively rapidly, and, except where expressly stated to the contrary, this ethereal salt was employed in all of our experiments. Ethyl butyrate further possesses the advantage that the amount split by water alone at the temperature at which we worked, is not measurable within the duration of our experiments. The experiments were carried out in test-tubes usually in the following manner: 4 cc. of water, 0.1 cc. toluene (as antiseptic), and 0.26 cc. ethyl butyrate[1] were placed in test-tubes. The tubes were then tightly corked and heated to the desired temperature, usually 40°, for five minutes, to bring their contents to the temperature of the bath, at the end of which time 1 cc. of the enzyme solution was added to each. The tube was then heated for the desired time, usually for fifteen minutes, when it was removed from the bath, plunged into ice-water, and titrated with N/20 KOH, neutral litmus being used as the indicator. In what follows, the initial acidity of the enzyme solution, usually amounting to 0.1 to 0.2 cc. N/20 KOH, has in every case been deducted from the cubic centimeters KOH required.

That the hydrolysis of ethyl butyrate and other ethereal salts was in reality brought about by the action of an enzyme, and not by other agents, was proved by numerous control experiments in which the solution of the enzyme was boiled before it was used. It may be of interest to note in this connection that in not a single instance did the control experiment show any hydrolysis of the ethereal salt.

The action of fat-splitting bacteria was prevented by antiseptics, usually toluene or thymol.

In our early experiments no special attention was paid to the amount of pancreas employed in the preparation of the extracts. It soon became manifest, however, that it would be

[1] At 30° C. (summer temperature) 0.26 cc. ethyl butyrate weighs 0.2300 gram. This quantity, if completely hydrolyzed, would require 39.7 cc. N/20 KOH. These numbers are employed in the following in calculating the amount of hydrolysis.

far better, for purposes of comparison at least, to employ definite amounts of the gland, and hence it will be observed in what follows that 10, 20, and even 50 per cent extracts were used. By this is meant that 10, 20, or 50 grams of gland were extracted with water and made up to 100 cc.

Several other tissues of the pig have been found to exhibit lipolytic activity towards ethyl butyrate, notably the liver, the submaxillary gland, and the kidney. A comparison of the lipolytic activity of 10 per cent extracts of these tissues has been made in the following manner: Tubes were prepared containing 4 cc. of water, 0.1 cc. toluene, and 0.26 cc. ethyl butyrate. These were then heated for five minutes at 40° when 1 cc. of the extract to be tested was added. The enzyme was allowed to act for forty minutes, at the end of which time the solution was titrated with N/20 KOH. The following were the results obtained:

	cc. N/20 KOH required.	Per cent of hydrolysis.
Pancreas	1.4	3.52
Kidney	0.7	1.76
Liver	4.1	10.29
Submaxillary gland	0.5	1.26

Taking the extract of the pancreas as unity, the lipolytic activity of the other tissues of the pig stand in the following ratio:

Pancreas	1.0
Liver	2.93
Kidney	0.50
Submaxillary gland	0.36

The liver of the pig showed such remarkable lipolytic activity that it occurred to us to compare it with the liver of certain other animals in this respect. Livers of the pig, beef, sheep, chicken, and duck were obtained in fresh condition in the market. Ten per cent aqueous extracts of these were prepared, and their activity toward ethyl butyrate tested in the manner already described.

Duration of experiments, fifteen minutes; temperature, 40°; antiseptic, toluene.

	cc. N/20 KOH required.	Per cent of hydrolysis.
Pig liver	3.45	8.66
Sheep "	1.90	4.77
Duck "	1.075	2.70
Beef "	0.875	2.20
Chicken "	0.775	1.95

Another interesting occurrence of lipase has been observed in the stomach of the pig. It has long been known that fats are hydrolyzed to some extent in the stomach.[1] The exact cause of this, however, seems not to have been determined with certainty. By some it is believed to be brought about by bacteria in the early stages of gastric digestion.[2] In order to test this point, 25 grams of the mucous membrane of the pig's stomach were ground with coarse sand, and water added. The extract was strained through linen cloth and made up to 100 cc. A small quantity of toluene was added as the antiseptic. This extract was found to possess lipolytic activity in neutral solutions. It was found to be rendered permanently inactive, however, by free hydrochloric acid in about the quantity normally present in gastric juice. These facts are brought out in the following experiments :

In the first series of experiments three solutions were employed, labeled A, B, and C, respectively. Solution A contained 5 cc. of 0.6 per cent hydrochloric acid, and 5 cc. of the active extract of thepig's stomach. The hydrochloric acid was, therefore, acting in a concentration of 0.3 per cent.

Solution B contained 5 cc. of the active extract of the pig's stomach and 5 cc. of water. Solution C contained 5 cc. of hydrochloric acid of 0.6 per cent and 5 cc. of water.

Experiments (First Series).

Time, 30 minutes ; temperature, 40°.

(1) 1 cc. solution A ; 4 cc. water ; 0.1 cc. toluene ; 0.26 cc. ethyl butyrate.

Increase in acidity = 0.05 cc. N/20 KOH = 0.13 per cent hydrolysis.

[1] Marcet: Proc. Roy. Soc., 9, 306 (1858); Ogata: Du Bois Arch., 515 (1881); Cash: Arch. f. Anat. u. Physiol., 1880, S. 323.

[2] Shäfer: "Text-book of Physiology," Vol. I, p.443 (1898).

(2) A duplicate experiment of above (No. 1) gave the same result. This slight hydrolysis was due to the hydrochloric acid present, see Exp. 4.

(3) 1 cc. of solution B ; 4 cc. water; 0.1 cc. toluene ; 0.26 cc. ethyl butyrate.

Increase in acidity = 0.25 cc. N/20 KOH = 0.64 per cent hydrolysis.

(4) 1 cc. solution C ; 4 cc. water ; 0.1 cc. toluene ; 0.26 cc. ethyl butyrate.

Increase in acidity = 0.05 cc. N/20 KOH, decomposition 0.13 per cent.

Experiments (Second Series).

Time, thirty minutes ; temperature, 40° C.

(1) 2 cc. extract of pig's stomach (boiled) ; 2 cc. water ; 0.1 cc. toluene ; 0.26 cc. ethyl butyrate.

Increase in acidity 0.1 cc. N/20 KOH ; decomposition 0.25 per cent.

(2) 2 cc. extract pig's stomach (boiled) ; 2 cc. 0.6 per cent hydrochloric acid ; 0.1 cc. toluene ; 0.26 cc. ethyl butyrate.

Increase in acidity 0.2 cc. N/20 KOH ; decomposition 0.5 per cent.

(3) 2 cc. active extract of pig's stomach ; 2 cc. 0.6 per cent hydrochloric acid ; 0.1 cc. toluene ; 0.26 cc. ethyl butyrate.

Increase in acidity 0.1 cc. N/20 KOH ; decomposition 0.25 per cent.

(4) 2 cc. active extract of pig's stomach ; 2 cc. water ; 0.1 cc. toluene ; 0.26 cc. ethyl butyrate.

Increase of acidity 0.8 cc. N/20 KOH ; decomposition 2 per cent.

These experiments certainly show that while a lipolytic enzyme is present in extracts of the mucosa of the stomach, it cannot effect the hydrolysis of fats in the presence of 0.3 per cent hydrochloric acid, and hence any hydrolysis of fats brought about by it in the stomach would probably be confined to the first stages of gastric digestion. The following experiments show further that this enzyme is rendered

permanently inactive by hydrochloric acid of this strength, proving, therefore, that on passing from the stomach into the intestine, the enzyme does not again become active.

Experiments (Third Series).

(1) 2 cc. active extract of pig's stomach; 2 cc. 0.6 per cent hydrochloric acid; 0.1 cc. toluene.

This mixture was heated ten minutes at 40°, when 0.2 cc. of litmus solution was added, and the contents of the tube just neutralized with N/20 KOH. 0.26 cc. ethyl butyrate was then added, and the tube again placed in the bath of 40°. The solution remained neutral permanently.

(2) Repetition of the above gave the same result.

(3) 2 cc. active extract pig's stomach; 2 cc. water; 0.1 cc. toluene.

This mixture was heated for ten minutes at 40°, when 0.2 cc. of litmus solution, 6.8 cc. water,[1] and 0.26 cc. of ethyl butyrate were added. The tube was then again placed in the bath at 40°. The solution became red in five minutes. After heating for twenty-five minutes, it required 0.7 cc. N/20 KOH; decomposition, 1.76 per cent. When returned to the bath, after neutralization, it became red again almost immediately. These experiments were repeated with extracts made from the stomach of a second animal with like results.

Still another occurrence of lipase, of doubtless great physiological interest, has been found in the small intestine of the pig. A short section of the intestine, including the duodenum and upper part of the jejunum was thoroughly cleansed in running water. At the time the animal was killed a small quantity of fluid was found in the small intestines. After cleansing, the mucosa was scraped off and macerated with coarse white sand and extracted with water. A 10 per cent extract was thus prepared. This extract was quite similar in appearance to the pancreatic extracts. Tubes were prepared containing 4 cc. of water, 0.1 cc. toluene, and 0.26 cc. ethyl

[1] This quantity of water was added in order to make the concentration the same as in Experiment 1.

butyrate. They were then heated in the bath at 40° for five minutes, at the end of which time 1 cc. of the active or boiled extract was added, after which the tubes were heated for fifteen or thirty minutes. The following results were obtained:

	Time in minutes.	cc. N/20 KOH required.	Per cent of hydrolysis.
1	15	0.9	2.26
2	30	1.65	4.14
3[1]	15	1.00	2.51
4	30	1.30	3.26

The boiled extract showed no activity whatever.

According to these results, the mucous lining of the small intestine possesses a lipolytic activity equal to three-fourths that of the fresh pancreas for equal amounts of tissue. The probable physiological significance of this fact will be briefly discussed in a subsequent part of this paper.

Effect of Filtration.

In our earlier work with the enzyme, the attempt was made to obtain it in the form of a clear solution, free from all suspended matter of any kind. By repeated filtration we succeeded in obtaining clear solutions, showing only a slight opalescence. Unfortunately, such solutions were always found to be exceedingly weak in lipolytic power. On the other hand, it was found that if the pancreas was thoroughly macerated with sand and the macerated mass thoroughly mixed with water, and simply strained through linen or cotton cloth, an extract of great lipolytic power was obtained. The effect of filtration was invariably to diminish the activity of the solutions. This was proved experimentally for aqueous and glycerin extracts of both the pancreas and liver. The same was also found to hold for the pancreatin of Parke, Davis & Co. A 2 per cent solution of this preparation is yellowish in color and is turbid. In this shape it possesses weak but measurable lipolytic power. On filtering a great many times a clear yellow solution is obtained, which possesses only a trace of its original lipolytic activity. To show the effect of filtration on the liver extract, the following experiments are cited: Before filtering, 1 cc. of a 10 per cent liver extract was found to

[1] In Experiments 3 and 4, 0.1 gram thymol was used as the antiseptic.

hydrolyze 6.28 per cent of ethyl butyrate in fifteen minutes at 40°. After filtering repeatedly through the same filter-paper, it hydrolyzed only 2.76 per cent of ethyl butyrate under the same conditions, showing a falling off in activity of over one-half as the result of filtration.[1] The removal of lipase, along with the suspended matter in the solution, was observed in still another connection. When an active pancreatic extract acts on ethyl butyrate at 40°, the butyric acid produced soon coagulates the proteids in the extract. On filtering, it was found that the clear filtrate possessed little or no lipolytic power, whereas the proteid coagulum was found to be exceedingly active. In view, therefore, of the injurious effect of prolonged filtration, it was deemed advisable to employ extracts which had been prepared in the manner above described, and which had simply been strained through linen or cotton cloth. Care was taken, however, to macerate thoroughly.

Stability of Lipase.

There seems to be a general impression among those who have worked with this enzyme that in order to obtain active pancreatic extracts they should be prepared within a few hours after the death of the animal.[2] It is true that, as compared with many enzymes, lipase is very unstable. That it does not disappear from the gland, however, as soon as is commonly supposed, is shown by the following experiments: Several pancreases were placed in cold storage, without antiseptic, on July 19, 1900. One cc. of a 10 per cent aqueous extract of a fresh pancreas on July 19, in 4 cc. of water with 0.26 cc. ethyl butyrate and 0.1 cc. toluene in fifteen minutes at 40°, required 2.45 cc. of N/20 KOH for neutralization. It, therefore, hydrolyzed 6.15 per cent of ethyl butyrate. On July 26 one of the pancreases was removed from the cold storage and a 10 per cent aqueous extract was prepared. It had an initial acidity of a 0.2 cc. KOH, and 1 cc. of it hydrolyzed 2.51 per cent of ethyl butyrate under the same condi-

[1] The same thing has been found to hold true in the case of other enzymes See Vines: Ann. of Bot., 1891. 499; Wortmann: Bot. Zeit., 1890, 37 et seq.; Brown and Morris: J. Chem. Soc. Trans. (1893). 604.

[2] Oppenheimer: "Die Fermente," 228, (1900); Shafer: "Text-book of Physiology," Vol. I, p. 339, (1897).

tions as the first. In seven days, therefore, at a temperature of 4°, there was a falling off in lipolytic activity of about 60 per cent. Still another pancreas in which putrefactive changes had begun, was observed to possess feeble lipolytic power. Still another preparation of the pig's pancreas throws light on the stability of lipase. The attempt was made to get rid of fat in the pancreatic extract by dissecting the gland as free from fat as possible, then grinding to a stiff paste with dry sand, and drying the resulting mass by exposing it in thin layers on filter-paper for several hours at ordinary temperature in the air. The dried mass was then extracted with ligroin and ddrie again in the air, and finally a small quantity of thymol was ground into the silicious mass to act as a preservative. An aqueous extract of the sand thus prepared was found to possess lipolytic activity, although it was much less active than the fresh aqueous extract of the pancreas. This preparation was made on July 12th. It was kept in the dark room in a glass-stoppered bottle. On August 17, an aqueous extract of the silicious mass was again made, and this was found to be nearly, if not quite, as active as it was originally. With proper precautions, the extracts of the pancreas and liver can also be preserved for some time without materially losing their activity. That such is the case may be seen from the following:

A 10 per cent aqueous extract of the pancreas was prepared on July 20th. The extract was placed in the refrigerator (temperature 1°), and kept there for several days, its activity being tested at intervals on ethyl butyrate in the following manner: 1 cc. of the enzyme with 4 cc. of water, 0.1 cc. toluene, and 0.26 cc. ethyl butyrate were heated together in a test-tube for fifteen minutes at 40°. at the end of which time titration was made with N/20 KOH.

The following results were obtained:

	N/20 KOH required. cc.	Per cent of hydrolysis.
Initial activity of extract	1.63	4.09
Activity of extract after forty-eight hours	1.77	4.44
Activity of extract after seventy-two hours	1.65	4.14

During seventy-two hours, the initial acidity of the extract had increased from 0.1 cc. to 0.25 cc. N/20 KOH. This was allowed for in the above calculations. Similar results have been obtained with the aqueous extracts of the liver of the pig. A 10 per cent aqueous extract was prepared on July 24th and kept on ice for several days in a glass-stoppered bottle. Its activity towards ethyl butyrate was tested at intervals, the experiments being carried out in test-tubes using 1 cc. of the enzyme solution, 4 cc. water, 0.1 cc. toluene, and 0.26 cc. ethyl butyrate. Temperature, 40°; duration of experiments, fifteen minutes.

	N/20 KOH required. cc.	Per cent of hydrolysis.
Initial activity of extract	1.7	4.26
Activity of extract after six hours	2.5	6.27
Activity of extract after forty-eight hours	2.4	6.03

The increase in the activity of these extracts on standing is interesting. The above are by no means the only cases in which such an increase in activity has been observed. The best explanation of this increase in activity on standing would seem to be that during the interval a certain amount of zymogen had been transformed to enzyme. It has also been observed that glycerin extracts of the pancreas are more stable than aqueous extracts of the same activity. Some idea of the stability of the glycerin pancreatic extracts may be gathered from the following observation: A glycerin extract was made on June 15th, 1900, and made use of in the investigation until June 23rd. Some of it was then preserved in sealed tubes in the dark room at summer temperature until August 18th, when it was still found to possess considerable lipolytic activity.

The Effect of Temperature on the Activity of Lipase.

It is well known that the activity of lipase is dependent on the temperature. According to Hanriot[1] the optimum temperature of the enzyme is 55°. At 60° he observed it to become considerably slower in its action, and at 72° it was found

[1] Compt. rend., **124**, 235 (1894).

to lose its activity altogether. Towards ethyl butyrate the activity of pancreatic lipase has been found to increase more or less regularly from 0° to 40° C. Above this temperature there is a gradual falling off in activity with rise of temperature. On exposure to a temperature somewhere between 65° and 70° for five minutes, a 10 per cent pancreatic extract loses its activity altogether. The activity of hepatic lipase varies much more with the temperature between 0° and 40° C. than that of the pancreatic enzyme as may be seen from the following experiments: Tubes containing 4 cc. of water, 0.1 cc. of toluene, and 1 cc. of a 10 per cent extract of the liver or pancreas were kept in baths at 40°, 30°, 20°, 10°, 0°, and —10° C., respectively, for five minutes in order to let them acquire the temperature of the bath. At the end of this time 0.26 cc. of ethyl butyrate was added to each tube, after which the tubes were allowed to remain in their respective baths thirty minutes, when they were placed in ice-water and titrated with N/20 KOH. The following results were obtained:

With the Pancreatic Extract.

Temperature.	N/20 KOH required. cc.	Per cent of hydrolysis.
40°	1.125	2.82
30°	1.26	3.16
20°	1.00	2.51
10°	0.75	1.88
0°	0.50	1.25

With the Hepatic Extract.

Temperature.	N/20 KOH required. cc.	Per cent of hydrolysis.
40°	4.5	11.29
30°	2.375	5.96
20°	2.10	5.27
10°	1.55	3.89
0°	0.9	2.26
—10°	0.28	0.70

Similar results have been obtained by Hanriot[1] for serum and pancreatic lipase. He found the latter, for example, to exhibit the same activity between 15° C. and 42° C., whereas

[1] Compt. rend., **124**, 778 (1897).

the serum lipase hydrolyzed twice as much monobutyrin at 42° C. as at 15° C. In this connection it is well to bear in mind that Schmiedeberg[1] seems to have made a distinction between the enzyme of the liver which splits hippuric acid, fats, etc, and which he calls histozyme, and ordinary pancreatic lipase. On the other hand, Nencki[2] has shown that the pancreas can also split hippuric acid.

On the Relative Stability of Different Ethereal Salts towards Lipase.

It is well known that other ethereal salts besides the fats are hydrolyzed in the intestine. Baas,[3] for example, found that, in the dog, salol was hydrolyzed in one case to the extent of 69 per cent and in another to 43.95 per cent. Methyl salicylate to 24.75 per cent and ethyl salicylate to 21.20 per cent. Heritsch[4] has also found that lipase could hydrolyze ethereal salts, for example, ethyl acetate. It occurred to us, therefore, that it would be interesting to measure the hydrolysis of a few of the commoner ethereal salts. Unfortunately, at this particular time only a limited number of these were on hand of sufficient purity for the purposes of the investigation. Ethyl formate, ethyl acetate, ethyl propionate, and ethyl butyrate were therefore selected for the experiments. Tubes were prepared containing 4 cc. of water, 0.1 cc. toluene, and 1 cc. 10 per cent pancreas extract. These were heated in the bath at 40° C. for five minutes. The ethereal salt to be tested was then added in the proportion of twice the molecular weight of the salt in milligrams. The tubes were then heated for fifteen minutes, when they were placed in ice-water and titrated with N/20 KOH. The following results were obtained:

	Quantity of ethereal salt used. Gram.	N/20 KOH required. cc.	Per cent of hydrolysis.
Ethyl formate	0.148	0.65	1.60
" acetate	0.176	0.37	0.93
" propionate	0.204	0.42	1.05
" butyrate	0.232	1.26	3.13

[1] Arch. f. Exp. Path. u. Pharm., **14**, 379 (1881).
[2] Arch. f. Exp. Path. u. Pharm., **20**, 367 (1886).
[3] Ztschr. f. physiol. Chem., **14**, 416 (1890).
[4] Centrbl. Med. Wis., 1875, 449.

A second series of experiments, exactly like the above, was tried, a more active pancreatic extract being used. The following results were obtained :

	N/20 KOH required. cc.	Per cent of hydrolysis.
Ethyl formate	0.7	1.75
" acetate	0.7	1.75
" propionate	1.15	2.87
" butyrate	1.75	4.37

Both series exhibit the same order of stability and both show that the higher the molecular weight of the acid, the more readily is its ethyl ester hydrolyzed by lipase. Ethyl formate is very easily hydrolyzed by pure water. This would probably account for the high values obtained with this compound.

In this connection it occurred to us that it might prove interesting, and perhaps valuable for future work upon this subject, to compare quantitatively the hydrolyzing power of certain acids with that of lipase upon ethyl acetate and ethyl butyrate. Hence, normal solutions of hydrochloric, acetic, and butyric acids were made use of in very much the same way as the enzyme in the experiments described in the above. Tubes were prepared containing 4 cc. of water and 0.176 gram of ethyl acetate. These were then heated in the bath at 40° C. for five minutes. One cc. of the normal acid was then run in and the tube heated for the desired length of time, at the end of which it was titrated with N/20 KOH. The following are the results obtained :

Acid.	Time. Minutes.	N/20 KOH required. cc.	Per cent of hydrolysis.
Water	15	0.05	0.13
	60	0.00	0.00
Hydrochloric	15	2.45	6.14
	30	4.45	11.15
Acetic	15	0.05	0.13
	60	0.4	1.0
Butyric	15	none	none
	60	"	"

Second Series with N-Hydrochloric Acid.

Time. Minutes.	N/20 KOH required. cc.	Per cent of hydrolysis.
15	2.45	6.14
30	4.45	11.15
45	6.10	15.29
60	8.15	20.43
90	10.75	26.95
120	14.05	35.21

Similar experiments on the hydrolysis of ethyl butyrate by acids gave the following results:

Conditions of experiment: Temperature, 40° C.; quantity of ethyl butyrate used in each experiment = 0.232 gram.

Acid.	Time. Minutes.	N/20 KOH required. cc.	Per cent of hydrolysis.
Hydrochloric	30	0.05	0.12
	60	0.8	2.00
	90	1.3	3.25
	120	1.5	3.75
Acetic	30	none	none
	60	0.1	0.25
	90	0.1	0.25
	120	0.3	0.75

Several interesting conclusions may be drawn from these results, the chief of which is the remarkable difference displayed by lipase and hydrochloric acid in effecting the hydrolysis of ethyl acetate and butyrate. Both pancreatic and hepatic lipase hydrolyze ethyl butyrate considerably more rapidly than the acetate, and this notwithstanding the fact that the latter is entirely in solution under the condition of the experiment, whereas the former is not. On the other hand, normal hydrochloric acid hydrolyzes the acetate about ten times as rapidly as the butyrate. As regards the cause of this remarkable difference between these two processes we are at present in the dark. It certainly offers an interesting field for further investigation.

On the Effect of Antiseptics on Lipase.

For certain parts of the work it was necessary to exercise considerable care in the choice of an antiseptic. It was,

therefore, thought desirable to study the effect of a number of the commoner antiseptics on this enzyme. Such a study seemed all the more desirable inasmuch as but few, if any, systematic studies of the action of antiseptics in general on enzymes have been undertaken. Some observers have studied the effect of one particular substance, and others that produced by another, but few, if any, general comparisons of the action of antiseptics have ever been made. The first series of experiments was made with salicylic acid. Tubes were prepared containing water (quantity indicated in the following table), 0.1 cc. of toluene, 1 cc. of 20 per cent pancreas extract, together with a certain amount of salicylic acid, varying in the amount from 0.1 to 1 cc. of a 0.5 per cent solution. The tubes were heated for five minutes at 40° C., when 0.26 cc. of ethyl butyrate was added. The tubes were then allowed to remain in the bath at 40° for fifteen minutes, when they were removed and titrated with N/20 KOH.

Quantity of water used.	Quantity of 0.5 per cent solution salicylic acid used.	N/20 KOH required.	Per cent of hydrolysis.
cc.	cc.	cc.	
4.0	none	2.25	5.65
3.9	0.1	2.12	5.32
3.5	0.5	1.4	3.51
3.0	1.00	0.2	0.50

This series exhibits a regular decrease in the activity of the enzyme in the presence of increasing amounts of salicylic acid.[1]

In the second series of experiments the following substances were employed in solution in the proportion of 1 to 1000: Silver nitrate, mercuric chloride, hydrocyanic acid, salicylic acid, thymol, and sodium fluoride. Tubes were prepared containing 4 cc. of water or of the solution of the antiseptic to be tested and 1 cc. of the enzyme (15 per cent pancreatic extract). The tubes were then heated for five minutes at 40°, at the end of which time 0.26 cc. of ethyl butyrate was added to each tube. The tubes were then allowed to remain in the bath at 40° for fifteen minutes, at the end of which time they

[1] An investigation of the power of salicylic acid to prevent the fermentation of wine must by Neubauer gave similar results. See J. prakt. Chem. (2), 11, 1.

were titrated with N/20 KOH. The following are the results:

Substance used.	N/20 KOH required. cc.	Per cent of hydrolysis.
Water	1.8	4.52
Hydrocyanic acid	1.7	4.27
Thymol	1.6	4.02
Mercuric chloride	0.3	0.75
Silver nitrate	0.15	0.38
Salicylic acid	none	none
Sodium fluoride	"	"

In the third series of experiments, the following substances were tried: Potassium cyanide, ammonium oxalate, and toluene. These substances were used in a solution containing 1 : 1000. Tubes were prepared containing 4 cc. of water, or of the solution of the antiseptic to be tested, and 1 cc. of pancreatic extract. These were heated at 40° for five minutes, when 0.26 cc. of ethyl butyrate was added to each tube. They were allowed to remain in the bath at 40° C. for fifteen minutes and then they were titrated with N/20 KOH. The following are the results:

Substance used.	N/20 KOH required. cc.	Per cent of hydrolysis.
Water	1.4	3.51
Ammonium oxalate	1.25	3.14
Toluene	1.15	2.89
Potassium cyanide	0.3	0.75

The effect of antiseptics 1 : 1000 was next tried on a 10 per cent hepatic extract, which had been made up originally without an antiseptic. Ethyl acetate was employed in this series of experiments. Tubes were prepared containing 3 cc. of water, 1 cc. of enzyme, and 1 cc. of the antiseptic. These tubes were then heated at 40° for fifteen minutes, after which 0.19 cc. ethyl acetate was added. The tubes were then allowed to remain in the bath at 40° for thirty minutes, when they were titrated with N/20 KOH. The following are the results:

Substance used.	N/20 KOH required. cc.	Per cent of hydrolysis.
Water	2.65	6.62
Toluene	2.90	7.25
Chloroform	2.50	6.25
Hydrocyanic acid	2.30	5.75
Mercuric chloride	2.00	5.00
Thymol	1.85	4.63
Silver nitrate	1.60	4.00
Salicylic acid	1.50	3.75
Osmic acid	1.45	3.63
Sodium fluoride	0.10	0.25

After titration the tubes were allowed to stand at ordinary temperature, 30° C., for an hour and a half, when they were titrated a second time, with the following results:

Substance used.	N/20 KOH required. cc.	Per cent of hydrolysis.
Water	3.02	7.55
Toluene	3.90	9.75
Chloroform	3.65	8.82
Hydrocyanic acid	3.40	8.50
Mercuric chloride	2.70	6.75
Thymol	3.00	7.50
Silver nitrate	2.20	5.50
Salicylic acid	3.05	7.60
Osmic acid	2.45	6.12
Sodium fluoride	0.20	0.50

Finally, the attempt was made to determine the effect of a number of antiseptics on liver and pancreatic extracts of about the same activity towards ethyl butyrate. Therefore, a 10 per cent liver extract was compared with a 20 per cent pancreatic extract under the following conditions: Tubes were prepared containing 3 cc. of water, 1 cc. of the antiseptic (1 : 1000) and 1 cc. of the enzyme. These were heated at 40° C. for five minutes to give the antiseptic a chance to act. Then 0.26 cc. ethyl butyrate was added and the tubes allowed to remain in the bath at 40° C. for fifteen minutes, when they were removed and titrated with N/20 KOH. The following are the results:

Series with 10 Per Cent Liver Extract.

Substance used.	N/20 KOH required. cc.	Per cent of hydrolysis.
Water	3.4	8.54
Formic aldehyde	3.7	9.29
Iodoform	3.5	8.78
Cocaine hydrochloride	3.5	8.78
Strychnine sulphate	3.45	8.66
Phenol	3.45	8.66
Toluene	3.3	8.28
Chloroform	3.25	8.16
Mercuric chloride	3.2	8.03
Bromoform	2.9	7.28
Thymol	2.9	7.28
Hydrocyanic acid	2.75	6.90
Salicylic acid	2.60	6.53
Silver nitrate	2.55	6.40
Osmic acid	2.00	5.02
Sodium fluoride	0.45	1.13
Hydrochloric acid	0.25	0.63
Hydrofluoric acid	0.25	0.63

Series with 20 Per Cent Pancreatic Extract.

Substance used.	N/20 KOH required. cc.	Per cent of hydrolysis.
Water	2.65	6.75
Bromoform	2.75	6.90
Chloroform	2.65	6.75
Hydrogen peroxide	2.50	6.27
Formic aldehyde	2.40	6.02
Salicylic acid	2.40	6.02
Thymol	2.30	5.77
Hydrocyanic acid	2.20	5.52
Iodoform	2.15	5.40
Mercuric chloride	2.15	5.40
Osmic acid	1.95	4.89
Strychnine sulphate	1.90	4.77
Toluene	1.80	4.52
Phenol	1.65	4.14
Silver nitrate	1.60	4.02
Hydrofluoric acid	0.50	1.25
Hydrochloric acid	0.40	1.00
Sodium fluoride	0.30	0.75

It is believed that in the last two series of experiments a mistake was made in not allowing the antiseptic to remain in

contact with the enzyme for a greater length of time in order that the effect of the weaker of them might have been more pronounced.

It will be observed that certain of the antiseptics tested exert the same effect on both the pancreatic and liver extracts; for example, chloroform is almost without effect, and thymol, hydrocyanic acid, and sodium fluoride each reduces the activity of the two extracts to the same extent. On the other hand, some of the substances used behave differently towards the two enzymes. For example, while strychnine sulphate and phenol both reduced the activity of the pancreatic extract about 30 per cent, they were without effect on the activity of the liver extract. On the other hand, osmic and salicylic acids seem to exert a more detrimental effect on the hepatic than on the pancreatic lipase. While perhaps not conclusive, these results are suggestive at least, for, if after repeated trials, it were to turn out that several substances exerted a different effect upon liver and pancreatic lipase, it would probably indicate that the two enzymes are in reality different substances. Upon pancreatic lipase especially, nearly all kinds of substances seem to exert a harmful effect. Towards a number of them, on the other hand, the lipase of the liver is more stable. Generally speaking, the more germicidal and antiseptic the substance, the greater its power to inhibit and destroy the activity of lipase. Substances like silver nitrate, mercuric chloride, salicylic acid, and osmic acid exert a more pronounced detrimental effect than the weaker antiseptic substances—such as toluene, chloroform, thymol, etc. One point of especial interest in this connection is the remarkably destructive action of sodium fluoride on lipase. In all of our tests this substance has been found to exert the most destructive influence upon lipase of any substance yet tried, unless it is hydrofluoric acid itself. The effect of acids in general has also been found to be very injurious.[1]

[1] Quite recently, in fact since this article was written, Kastle and Hardin have been engaged in studying the toxic action of different acids on lipase. It is their intention to present the results obtained in the form of a separate communication. It may not be out of place to state in this connection, however, that it has been found in general, that the toxic effect of acids upon lipase is proportional to their strength.

On the Effect of Concentration of the Enzyme on the Velocity of the Reaction.

The attempt has been made to determine the effect of concentration on the activity of hepatic and pancreatic lipase. According to some observers, the rate of hydrolysis by lipase is proportional to the concentration of the enzyme, especially if the conditions are those most favorable to the reaction. In order to determine the effect of concentration of the enzyme on its activity, concentrated extracts, usually 50 per cent, of the pancreas and liver were prepared, and from these the more dilute extracts, such as 5, 10, 20, and 40 per cent, respectively, were made by dilution. Tubes were then prepared containing 4 cc. of water, 0.1 cc. of toluene, and 0.19 cc. of ethyl acetate. The tubes were heated for five minutes at 40° C., at the end of which time 1 cc. of the enzyme of a given concentration was then added, after which the tubes were heated for thirty minutes. They were then removed from the bath and titrated with N/20 KOH. The following results were obtained :

Concentration of the pancreatic extract. Per cent.	N/20 KOH required. cc.	Per cent of hydrolysis.
5	0.13	0.33
10	0.25	0.625
20	1.08	2.70
40	2.00	5.00

Concentration of the hepatic extract. Per cent.	N/20 KOH required. cc.	Per cent of hydrolysis.
5	0.8	2.00
10	1.35	3.37
20	3.88	9.70
40	6.90	17.25

Still another series, with an extract which was prepared from the pig's liver, that had stood for twenty-four hours on ice, gave the following results :

Conditions of the experiments the same as in the first two series.

Concentration of the hepatic extract. Per cent.	N/20 KOH required. cc.	Per cent of hydrolysis.
5	1.2	3.00
10	2.02	5.05
20	2.93	7.32
40	4.50	11.25

These results leave no room for doubt that the rate of change increases with the quantity of enzyme present in the solution, in fact in one of the above series, *viz.*, that of the liver extract it is approximately proportional to the concentration of the enzyme. The fact, however, must be borne in mind in the interpretation of the above results, that the fatty acid produced in the hydrolysis of the ethereal salt soon coagulates the proteid present in the solution, and, as has already been pointed out, this coagulation removes the lipase more or less completely from solution; and while the coagulum still possesses considerable lipolytic power, it is doubtful if the enzyme acts to as great advantage as when in solution. We have not yet had the opportunity of comparing the activity of the coagulum with that of the original extract. It is a factor, however, which might very materially vitiate the results and should certainly be taken into consideration.

On the Effect of the Concentration of the Ethereal Salt on the Velocity of the Reaction.

The hydrolysis of an ethereal salt by lipase certainly presents some analogy at least to the hydrolysis of such a compound by an acid. On account of the instability of the lipase itself, however, its action rather resembles that of the hydrolysis of an ethereal salt by an acid itself so unstable as to be constantly undergoing a gradual decomposition during the change. The question therefore suggests itself in this connection, Is the amount of hydrolysis for any given interval, proportional to the active mass of the ethereal salt? Hanriot and Camus[1] have observed that the rate of hydrolysis varies

[1] Compt. rend., **124**, 235 (1897).

slightly with the quantity of ethereal salt present. In order to test this point further the equation for a reaction of the first order $\frac{1}{\theta} \log \frac{A}{A-x} = k$ was applied to the reaction in hand. Tubes containing 4 cc. of water, 0.1 cc. toluene, and 0.26 cc. ethyl butyrate were prepared and heated for five minutes at 40° C. One cc. of a 10 per cent extract was then added and the tubes heated for the desired length of time and titrated with N/20 KOH. The following results were obtained:

Time. Minutes.	N/20 KOH required. cc.	Per cent of hydrolysis.	k.
5	2.60	6.53	0.01354
10	3.45	8.66	0.00907
15	3.40	8.53	0.00592
20	3.80	9.54	0.00500
25	4.25	10.67	0.00500
30	3.75	9.41	0.00329
60	6.90	17.32	0.00316
120	10.10	25.35	0.00244
180	11.3	28.36	0.00184

One Minute Series.—Conditions the same as in the preceding.

Time. Minutes.	N/20 KOH required. cc.	Per cent of hydrolysis.	k.
1	1.3	3.26	0.03344
2	1.8	4.52	0.02296
3	1.95	4.89	0.01657
4	2.30	5.77	0.01481
5	2.60	6.53	0.01354

The following series was tried to show the effect of varying the amount of ethyl butyrate. The tubes contained 4 cc. of water, 0.1 cc. toluene, and 1 cc. of the 10 per cent liver extract used in the preceding series. The tubes were heated at 40° C. for five minutes, at the end of which time a certain quantity of ethyl butyrate was added and the tubes heated for five minutes at 40° C., and then titrated. The following results were obtained:

Quantity of ethyl butyrate employed.		N/20 KOH required.	Quantity of ethyl butyrate hydrolyzed.
cc.	Gram.	cc.	Gram.
0.065	= 0.0575	2.1	0.0122
0.13	= 0.1150	2.7	0.0156
0.195	= 0.1725	2.6	0.0151
0.26	= 0.2300	3.2	0.0185

According to these results, the actual amount of ethyl butyrate hydrolyzed by the enzyme in a given time, is very largely independent of the concentration of the ethereal salt. It was thought possible that the fact, that in all of these experiments a certain amount of the butyrate remained undissolved, might offer an explanation of these results. It was thought desirable, therefore, to repeat some of the work on the concentration of the ethereal salt, using ethyl acetate in the place of ethyl butyrate, for the reason that the former is quite soluble in water. A solution of ethyl acetate was prepared containing 0.01414 gram of the compound per cubic centimeter. Forty cc. of this solution was placed in a glass-stoppered flask, together with 0.5 cc. toluene, and heated in the bath at 40° C. until it had reached the temperature of the bath. Ten cc. of 10 per cent liver extract, which had also been warmed to the temperature of the bath, were then added, and the time of the experiments was reckoned from this instant. The flask was constantly shaken, and at certain intervals 5 cc. of the mixture were withdrawn from the flask by means of a pipette and titrated with N/20 KOH. The results are given below:

Time. Minutes.	N/20 KOH required. cc.	Per cent of hydrolysis.	*k*.
5	0.45	3.50	0.00714
10	0.75	5.83	0.00603
15	0.90	6.99	0.00484
20	1.00	7.77	0.00404
30	1.30	10.10	0.00355
45½	1.65	12.82	0.00303
60	1.85	14.37	0.00258

The effect of varying the concentration of the ethyl acetate was tried in the following manner: Ten per cent extract of liver used. Temperature, 40° C. Time, fifteen minutes.

Substance used in experiment.	N/20 KOH required. cc.	Quantity of ethyl acetate originally present. Gram.	Quantity of ethyl acetate hydrolyzed. Gram.
4 cc. ethyl acetate solution and 1 cc. of enzyme	0.9	0.05656	0.003952
3 cc. ethyl acetate solution, 1 cc. water, and 1 cc. enzyme	0.875	0.04242	0.003861
2 cc. ethyl acetate solution, 2 cc. water, and 1 cc. enzyme	0.9	0.02828	0.003952
1 cc. ethyl acetate solution, 3 cc. water, and 1 cc. enzyme	0.775	0.01414	0.003403

It will be observed that, as in the case of ethyl butyrate, the hydrolysis of ethyl acetate by lipase is largely independent of the concentration of the ethereal salt.

In this connection the attempt has also been made to determine whether the hydrolysis of ethyl butyrate by lipase is complete. With this in view, two bottles were prepared as follows:

No. 1 contained 40 cc. of water, 10 cc. of 10 per cent pancreatic extract, 1 cc. toluene, 0.65 cc. ethyl butyrate.

No. 2 contained 40 cc. of water, 10 cc. of 10 per cent hepatic extract, 1 cc. toluene, 0.65 cc. ethyl butyrate.

These were allowed to stand at ordinary temperature for several days. At intervals during this time, 5 cc. of the contents were withdrawn by means of a pipette and titrated with N/20 KOH. The following results were obtained:

	Time. Hours.	N/20 KOH required. cc.	Per cent of hydrolysis.
5 cc. No. 1 (pancreatic) lipase	19	3.5	35.0
5 " " " "	19	3.5	35.0
5 " " " "	47	4.3	43.0
5 " " " "	47	4.15	41.5

				Time. Hours.	N/20 KOH required. cc.	Per cent of hydrolysis.
5 cc.	No. 1	(pancreatic)	lipase	137	4.3	43.0
5 "	"	"	"	137	4.3	43.0
5 "	No. 2	(hepatic)	"	19	7.1	71.0
5 "	"	"	"	19	7.25	72.5
5 "	"	"	"	47	9.35	93.5
5 "	"	"	"	47	9.55	95.5
5 "	"	"	"	47	9.4	94.0
5 "	"	"	"	137	9.2	92.0

After titration these tubes were allowed to stand for a time. In every case the contents of the tube became acid in reaction, thereby indicating that active enzyme was still present in the solution. The above results are such as to indicate that except when relatively large amounts of the enzyme are employed, the reaction is incomplete. Whether with lipase of different origin or with varying amounts of the same enzyme the limit reached in all cases would be the same is a matter which can only be settled by further investigation. The fact that the hydrolysis is not complete under ordinary circumstances is in keeping with the more or less regular falling off in the velocity of the reaction described in the above, and is probably to be explained by the fact that the hydrolysis of an ethereal salt by lipase is a reversible process, and that the enzyme, as a catalytic agent, is concerned chiefly in bringing about a condition of chemical equilibrium between the ethereal salt and water and the products of the hydrolysis. Acids also have been found to greatly inhibit the activity of the enzyme, so that the great falling off in the rate of hydrolysis is doubtless, to some extent at least, assignable to this cause.

The Reversibility of the Action of Lipase.

In spite of the vast amount of work which has been done upon the enzymes, we possess at present no unifying conception as to the manner of their action. For the solution of this problem, we must probably look to the study of the kinetics of enzyme action, and one of the first questions to present itself

in this connection is, Are the enzymes reversible in their action? If so, then an accumulation of the products of their action should have an increasingly inhibiting effect on the further progress of the change, until finally equilibrium would be established between the initial substance and the products of the change.

O'Sullivan and Thompson,[1] in their exhaustive study of the action of invertase on cane-sugar, could not observe any inhibiting effect from the presence of the products of the change. On the other hand, Sheridan Lea[2] found the digestion of starch by salivary diastase to be more rapid and much more complete when the products of the digestion were removed by dialysis than when the digestion was performed under identical conditions, but without this precaution. He obtained similar results in his investigation of tryptic digestion. As to the effect of peptone on peptic digestion, the views are somewhat contradictory. Kühne[3] states that peptone has a retarding effect on peptic digestion, while the opinion of Chittenden and Ely[4] contradicts this. Tamman,[5] from his studies of this phase of the problem, arrived at the following conclusions:

1. Hydrolyses produced by enzymes are incomplete, especially if the quantity of material acted on is large (invertase and rennin are exceptions to this).

2. The incompleteness of the action is due to the accumulation of the products of the fermentation.

3. Removal or dilution of these products, or elevation of the temperature, allows the fermentation to continue.

It would appear that the results obtained by Tamman are easily explicable on the supposition that the enzymes are reversible in their action, the reaction ceasing when the products of the hydrolysis reach a definite concentration in consequence of equilibrium being established. As Tamman failed to get any indication experimentally that the enzymes are capable of effecting the synthesis of the substances they are

[1] J. Chem. Soc., **57**, 926 (1890).
[2] Jour. of Physiology, **11**, 226 (1890).
[3] Lehrbuch d. Phys. Chemie, 1866, S. 39.
[4] Jour. of Physiology, **3**, 327.
[5] Ztschr. physiol. Chem., **16**, 271 (1892); Ztschr. physikal. Chem., **28**, 426 (1895).

capable of hydrolyzing, he was forced to explain his results in another way. He advanced the view that the products of the hydrolysis act on the enzyme, transforming it into an inactive modification, and that on removal of these products it is converted into the active modification again.

Oppenheimer,[1] accepting Tamman's results as final, sought to supply a unifying conception of the action of enzymes. He believes the enzymes are capable of inducing only exothermic reactions—that they are only capable of setting free potential energy, and that none of the endothermic, that is, synthetic processes taking place within the organism, can be classed as enzymic, but must be attributed to the peculiar activity of living protoplasm.[2] He admits, however, that this view is completely at variance with the recent work of Hill,[3] who has proved that the action of maltase on maltose is reversible,—first, by showing that maltase can effect the synthesis of maltose from glucose, and, secondly, by showing that the action of maltase on maltose tends to an equilibrium, which is the same whether we start with glucose or an equivalent quantity of maltose.

It is believed that the results reached in this investigation of lipase throw still further light on the subject and greatly strengthen the belief that all enzyme action would probably prove to be reversible if proper conditions of concentration, etc., could be attained.

In many instances, however, it will probably not be practicable to secure the proper conditions, for as in the rennin coagulation of caseinogen solutions, one of the products of cleavage seems to undergo instantaneous rearrangement in the presence of soluble calcium salts, and is thereby removed from the field of activity, thus rendering reversibility impossible. Then again, in some cases, for example, the action of invertase on cane-sugar, the reaction seems to be so nearly complete in one direction that it is doubtful whether reversibility could be demonstrated experimentally. While the catalysis of ethers by strong acids is practically complete, still these are typical reversible reactions. In attempting to determine whether lipase is reversible in its action, it was decided to test its power to ef-

[1] "Die Fermente und ihre Wirkungen," Leipzig, 1900, S. 55.
[2] The same view has been expressed by Nasse. Maly's Jahrsbr., 1894. S. 718.
[3] J. Chem. Soc., 73, 634 (1898).

fect the synthesis of ethyl butyrate, inasmuch as this substance possesses the following advantages over a true fat: First, it possesses such a characteristic odor that this alone serves as a very delicate and unmistakable test for its presence. Secondly, in order to effect the synthesis of a fat, it would be necessary, or at least highly desirable, to have the extract of the enzyme free from fat. Up to this time we have not been able to prepare a pancreatic extract free from fat without greatly diminishing its lipolytic power. In the synthesis of ethyl butyrate, however, it would not be necessary to remove the fat, and hence it would be possible to employ the most active pancreatic extract obtainable. Finally, a demonstration of the power of lipase to effect the synthesis of ethyl butyrate would be as conclusive as the synthesis of a fat for the reason that the compounds are exactly analogous from a chemical point of view.

Experiment 1.—5 cc. N/100 butyric acid, 2 cc. of 13 per cent alcohol, and 1 cc. of diluted glycerin extract of pig pancreas were sealed in a test-tube, and heated at 48°.5 for thirty-six hours. When opened it had distinctly the odor of ethyl butyrate.

Experiment 2.—A mixture of 180 cc. of N/40 butyric acid, and 72 cc. of 30 per cent alcohol was divided into two equal parts. To part 1, 25 cc. of a 10 per cent glycerin extract of pig pancreas was added; 25 cc. of the same glycerin extract which had been brought to boiling, and then cooled, was added to part 2. The flasks were left open at ordinary temperature. In a few minutes the flask containing the fresh enzyme had developed a decided odor of ethyl butyrate. The flask was then corked and allowed to stand a week when the odor of ethyl butyrate was still perceptible. The flask containing the boiled enzyme was also corked and was allowed to stand a week under the same conditions. At no time could the odor of ethyl butyrate be detected in the control experiment. Exactly similar experiments were carried out in the presence of toluene water with similar results. Two flasks were also made up, each containing 0.25 gram of β-naphthol. Here, also, the odor of ethyl butyrate was

apparent in the flask containing the active enzyme, while in the flask containing the boiled enzyme there was never the faintest odor of the ethereal salt. Similar results were obtained with glycerin extracts of the cow pancreas. In these experiments, the only test for the production of the ethereal salt was the odor, and although this is very delicate and unmistakable, it seemed desirable to obtain, if possible, larger quantities of the substance. With this in view, the following experiment was made :

Four hundred grams of pig pancreas (which had been removed from the animal one and a half hours) was thoroughly ground up with sand and extracted with water, the extract being made up to 2000 cc. This was divided into two parts of 1000 cc. each. One part was placed in a balloon flask (A) of 4 liters capacity, while the other part was boiled for three minutes. After cooling, enough water was added to supply that lost in boiling. It was then placed in a flask (B) similar to (A). To each of these flasks 2 grams of powdered thymol were added as antiseptic. Each of the flasks was provided with a three-hole rubber stopper, through which passed : 1. A separating funnel ; 2. A tube reaching to the bottom of the flask with a small aperture at the end and connected with an apparatus for giving a current of air ; and 3. A tube connecting the flask with a condenser. A distilling bulb, with its delivery tube drawn out to a capillary opening, and packed in ice, was used as a receiver. The inner tube of the condenser extended to the bottom of the receiver, and the connection between receiver and condenser was made tight with rubber tubing. 3800 cc. of practically N/10 butyric acid (5 cc. = 9.2 cc. N/20 KOH) was made up. This was divided into two equal parts, and to each part was added 100 cc. of 95 per cent alcohol and 4 grams of powdered thymol. One part of this mixture was added to flask (A) and the other simultaneously to flask (B). It was allowed to flow into the flasks very slowly through the separating funnels, four and a half hours being consumed in the operation. The flasks were kept in water-baths at laboratory temperature, 23° to 27°, and both under identical conditions, with respect to sunlight, etc. The initial acidity of the boiled and unboiled extract was the same,

viz., 1 cc. = 0.3 cc. N/20 KOH. The initial activity of the enzyme was taken, and found to be high. In a tube containing 4 cc. of water, 1 cc. of extract, 0.26 cc. ethyl butyrate, and 0.1 cc. toluene, and heated at 40° C. for fifteen minutes, 6.45 per cent of the ethyl butyrate was hydrolyzed. The flasks were allowed to stand forty hours with occasional shaking, both flasks being shaken equally. In order to separate any ethyl butyrate, which might have been produced, the flasks were then heated on the water-bath, the distillation being carried on in a slow current of air. About 25 cc. of the distillate were collected in each case.[1] The odor of the distillate from flask (A) indicated plainly and unmistakably the presence of ethyl butyrate; it also contained some alcohol and butyric acid, but the odor of these could not be detected on account of the butyric ether. In the distillate from the blank (B), the presence of even a trace of the ether was considered very doubtful by all who examined it, while the odor of alcohol and butyric acid were distinct. The distillate obtained from (A) was redistilled from a water-bath (this time no current of air was used), and 5 cc. of a clear distillate was collected. This was still faintly acid with butyric acid and contained some alcohol.

A small portion of this distillate was reserved for experiment and the remainder was poured into water. The mixture at once became turbid and a light ethereal oil rose to the surface. Altogether about 1 cc. of impure ethyl butyrate was obtained in this experiment. Hence about 5 per cent of the butyric acid present had been transformed into the ethereal salt. This quantity was too small for purification and hence no analysis of it was attempted. Its characteristic odor, however, together with its other properties, and its mode of formation left no room for doubt regarding its composition. A small amount of it was saponified with caustic soda, and the solution evaporated to dryness. On adding sulphuric acid to the residue the odor of butyric acid was perceptible at once.

[1] Previous experiment had shown that on distilling a mixture of ethyl butyrate and a large amount of water, practically all the ethereal salt passes over in the first portions of the distillate.

The first distillate from flask (B), *viz.*, that obtained in the control experiment, was treated in exactly the same manner as that from flask (A), *i. e.* the first distillate was distilled a second time and the first 5 cc. of distillate, No. 2, collected. This second distillate had only a trace of the odor of ethyl butyrate, and when poured into water it dissolved without turbidity and no ethereal oil separated on standing. The conduct of the second distillate from (A) and (B) towards lipase was also tested. Tubes were prepared containing 4 cc. of water, 1 cc. of enzyme, 0.1 cc. toluene, and 0.26 cc. of the second distillate from (A) or (B).

The following results were obtained:

With hepatic extract; time, 15 minutes; temperature, 40° C.

	N/20 KOH required. cc.
(1) 2nd distillate (A)	0.65
(2) " " (A)	0.70
(1) " " (B)	none
(2) " " (B)	0.05

With pancreatic extract; time, 50 minutes; temperature, 40° C.

	N/20 KOH required. cc.
(1) 2nd distillate (A)	0.65
(2) " " (B)	none

Control experiments on the second distillate from (A) using the boiled enzyme, showed no hydrolysis even after long standing. These results prove the presence of an ethereal salt in the second distillate from (A), but none, or at best only traces thereof, in the second distillate from (B).

From these experiments it would seem that lipase is reversible in its action upon ethyl butyrate at least.

The fact that lipase is reversible in its action will doubtless afford an explanation of a number of somewhat obscure physiological processes. For example, Schmiedeberg[1] has shown that the kidney of the pig is capable of splitting hippuric acid into benzoic acid and glycocol, and that it is also capable of effecting the synthesis of hippuric acid from these substances.

[1] Arch. f. Exp. Path. and Pharm., 14, 379 (1881).

He attributes the splitting of the hippuric acid to a soluble enzyme which he calls histozyme, and which he succeeded in extracting from this organ, and also from the liver. On the other hand, he believes the synthesis of the hippuric acid to be due to the absence of histozyme, and to be induced by some other agency. According to him, the processes of decomposition and synthesis going on in the living organism, are brought about by entirely different agencies. The formation of hippuric acid being a reversible reaction, however, it would seem more probable that the histozyme simply has the power to hasten the establishment of the equilibrium between benzoic acid and glycocoll. According to this view, if benzoic acid and glycocoll are passed through the kidneys, a certain quantity of hippuric acid would be built up under the influence of the histozyme, whereas if hippuric acid itself were passed through this organ a certain quantity of it would be hydrolyzed by the histozyme. The fact that lipase can effect the synthesis of an ethereal salt, like ethyl butyrate, will doubtless throw some light on two exceedingly interesting and important physiological processes, *viz.*, the absorption of fats and the ripening of fruits. Without entering minutely into the physiological aspects of fat-absorption, it may be observed in passing that serious objections have been urged against the various theories which have been put forward from time to time to account for the absorption of fats in the intestinal tract. It has been pointed out by Rachford[1] that the pancreatic juice is capable of hydrolyzing all the fat of a fatty meal in the period of pancreatic digestion. In the living intestine, therefore, the hydrolysis should be complete, inasmuch as the removal of the products of the hydrolysis by absorption prevents the establishment of equilibrium. On the other hand, the products of the hydrolysis in their transition through the epithelial cells come in contact with a lipolytic enzyme, the presence of which in these cells has been demonstrated in the above (see p. 493). The lipase now finds itself in contact with only fatty acid and glycerin, and hence in acting catalytically to bring about the chemical equilibrium, it effects the synthesis of a fat. This would offer a satisfactory explanation of the presence of fat

[1] Jour. Physiology, **12**, 92 (1891).

granules in these cells. As the fatty acid and glycerin diffuse out of these cells through the basement membrane, the fat in these cells would speedily disappear were it not that these substances were constantly being absorbed from the lumen of the intestine. When absorption ceases, however, the fat present in the epithelial cells is at once hydrolyzed by the lipase present. This hydrolysis is in all probability complete for the reason that the products of the hydrolysis, *viz.*, glycerin and fatty acid, are being constantly removed by diffusion. According to this view, therefore, no fat ever enters or leaves the epithelial cells as such, but as fatty acid and glycerin.

These two substances then enter the central lacteal, where equilibrium is again established and there is a large production of fat. This view derives support from the fact that fats and soaps exist side by side in the blood[1] in the serum of which Hanriot[2] has demonstrated the presence of a very powerful lipolytic enzyme. It is our intention to look for a lipolytic enzyme in the lymph and lymphatic tissue when the opportunity presents itself.

As is well known, lipase is by no means confined to the animal organism. It has been found by numerous observers in the resting and germinating seeds of various plants, but hitherto it has been looked upon as being concerned solely with the splitting of fats, especially during the period of germination.

On the other hand, Sachs[3] long ago pointed out that "the presence of fats in the seedling can only be explained by assuming that glycerin and the fatty acids travel from cell to cell, and are continually becoming re-united for the formation of fat," and quite recently Charabot[4] has also reached the conclusion that, in the plant, ethereal salts result from the direct action of the free acids on the alcohols. In view of these facts and of the results described in the above, it would seem logical to conclude that lipase can effect the formation as well as the decomposition of fats and ethereal salts in the plant, and that this ferment plays an important part not only in the utili-

[1] Hoppe-Seyler: Ztschr. physiol. Chem., **8**, 503.
[2] Compt. rend., **123**, 753 (1896).
[3] Sachs' "Plant Physiology," 1887, p. 347.
[4] Bull. Soc. Chim. [3], **23**, No. 5.

zation and translocation of these important reserve materials, but also in the storing up of these substances in the seed and fruit.

Summary of Results.

The results reached in this investigation may be briefly summarized as follows:

1. It has been found that ethyl butyrate is so easily hydrolyzed by lipase as to make it a very useful substance in the investigation of a number of problems connected with the chemical action of this enzyme.

2. By means of ethyl butyrate it has been possible to prove the presence of lipase in a number of organs and tissues in the animal organism, notably in the liver, stomach, and small intestine.

3. It has been shown that lipase is almost completely removed from its solutions by repeated filtration at ordinary pressure.

4. It has also been shown that lipase is a much more stable enzyme than has hitherto been supposed.

5. It has been shown that animal lipase hydrolyzes ethereal salts most rapidly at 40° C., and that at 65° to 70° C. the enzyme is destroyed.

6. The conduct of lipase towards a few members of an homologous series of ethereal salts has been studied. It has been found that the stability of these ethereal salts towards lipase decreases with increase in molecular weight of the combined acid. The converse of this was found to be true for the hydrolysis of these ethereal salts by acids.

7. The effect of about twenty of the commoner antiseptics on lipase has been studied. By far the greater number of these substances have been found to exert a harmful effect on the enzyme. This was found to be particularly true of sodium fluoride and hydrofluoric acid and acids generally.

8. A study of the kinetics of the reaction has given the following results:

First, the velocity of the reaction is not proportional to the active mass of the ethereal salt. Secondly, the velocity of the reaction is nearly proportional to the concentration of the en-

zyme. Thirdly, the reaction is incomplete. This holds for all ordinary concentrations of the enzyme and the ethereal salt. With very concentrated or energetic extracts of the lipase or with very small amounts of ethereal salt the hydrolysis certainly approaches completion. Finally, the coefficient of velocity as calculated according to the equation for a reaction of the first order, is not constant, but shows a regular falling off as the reaction proceeds.

9. By means of lipase we have effected a synthesis of ethyl butyrate from butyric acid and alcohol, thereby proving that the hydrolysis of an ethereal salt as effected by this enzyme is a reversible reaction. Finally, the bearing of this result on fat absorption and the storing up and translocation of fatty reserve material in the plant has been briefly discussed in the above.

STATE COLLEGE OF KENTUCKY,
LEXINGTON, Sept., 1900.

DERIVATIVES OF PHENYL ETHER.

BY A. N. COOK AND H. W. HILLYER.

Up to the present time diphenyl ether and its derivatives have been prepared by at least thirteen different methods and have been studied by no less than twenty-five different chemists. While the methods are numerous the yield in almost every case is remarkably small. The leading methods that have been used are as follows:

Diphenyl ether was prepared by Hofmeister[1] by the action of diazobenzene sulphate on phenol. The yield was small. By using the chloride instead of the sulphate and changing the conditions Hirsch[2] obtained a yield of 50 per cent of the aniline used.

Mertz and Weith[3] obtained a small yield of diphenyl ether by heating phenol with zinc chloride and also with aluminium chloride.

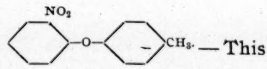
Gladstone and Tribe[4] made various methyl phenyl ethers by distilling the corresponding aluminium cresolates.

[1] Ber. d. chem. Ges., **3**, 747.
[2] *Ibid.*, **23**, 370.
[3] *Ibid.*, **14**, 187.
[4] J. Chem. Soc. (London), **41**, 5; and **49**, 27.

Willgerodt[1] obtained a large yield of the trinitro derivative by heating picryl chloride and phenol in alcoholic solution with one molecular equivalent of potassium hydroxide.

One of the most productive methods which has been used is that originated by Haeusermann and Teichmann,[2] and also independently by F. Ullmann.[3] They heated potassium phenolate and several of its derivatives with various nitrohalogen derivatives of benzene and obtained, as a rule, good yields of nitro derivatives of phenyl ether. In approaching the study of phenyl ether derivatives this method was adopted and is here extended to reactions with the cresols.

For clearness in designation the nomenclature used by Haeussermann and Bauer[4] has been adopted.

2-Nitro-4'-methyl Phenylether, 

— This compound was prepared by the action of *o*-bromnitrobenzene on potassium *p*-cresolate,[5] the reaction taking place according to the following equation :

$$NO_2C_6H_4Br + KOC_6H_4CH_3 = NO_2C_6H_4OC_6H_4CH_3 + KBr.$$

One part by weight of potassium *p*-cresolate was heated in a small Florence flask, on a bath of fusible metal, with three parts of *o*-nitrobrombenzene to a temperature of 125° to 130°, when a vigorous action began, accompanied by a rise of temperature of several degrees. As soon as the action had ceased, which required about five minutes, the molten mass was cooled and extracted with ether. The ether extract was washed with a solution of potassium hydroxide to remove any free cresol which might be present. The excess of bromni-

[1] Ber. d. chem. Ges., **12**, 1278.

[2] *Ibid.*, **29**, 1446.

[3] *Ibid.*, **29**, 1878.

[4] *Ibid.*, **29**, 2083.

[5] The potassium cresolate was made by treating one part of paracresol with a molecular equivalent of potassium hydroxide dissolved in one part of water, evaporating to dryness on the water-bath with continual stirring and then drying in the air-bath at 100° C. for half an hour. This method yielded a very good product, which was of a slightly yellow color. An endeavor to prepare the cresolate by dissolving metallic potassium in the cresol, according to the method used by Haeussermann and Teichmann, and by Ullmann in working with phenol, yielded a dark, tarry product, which very materially effected the yield and purity of diphenyl ether made from it.

trobenzene was distilled off with steam, and the phenyl ether distilled under diminished pressure. Under a pressure of 25 mm. the substance boils at 220° C. The yield was one gram of the ether for every gram of the cresol used. On crystallizing it several times from alcohol it was completely purified, and on analysis yielded the following results :

	Calculated for $NO_2C_6H_4OC_6H_4CH_3$.	Found. I.	II.
C	68.04	68.12	68.20
H	4.83	4.75	4.77
N	6.11	6.28	6.32

The compound melts at 49°. It distils with partial decomposition at ordinary pressure. It is not volatile with steam. It is very soluble in hot alcohol, but much less soluble in cold alcohol, from which it crystallizes in beautiful sulphur-yellow and apparently monoclinic crystals of considerable size. It is very soluble in ether, acetic acid, chloroform, benzene, and other organic solvents. It is sparingly soluble in petroleum ether. It is dissolved by concentrated sulphuric acid with slight charring and by concentrated nitric acid, which apparently oxidizes it.

2-Nitrophenylether-4'-carbonic Acid, $NO_2C_6H_4OC_6H_4COOH$. —This acid was prepared by dissolving the above-mentioned ether in glacial acetic acid, heating on the water-bath, and adding very slowly a cold solution of chromium trioxide in glacial acetic acid until a test portion failed to become turbid on diluting with a large amount of a dilute solution of sodium hydroxide. To accomplish this, three or four times the theoretical amount of chromium trioxide was necessary, a part of the acid formed being oxidized before all of the methyl compound was changed to acid. When the oxidation was judged to be complete, the acid was precipitated from the acetic acid solution by diluting with a large amount of water. It was purified by washing with water, dissolving in dilute ammonia, filtering, reprecipitating with hydrochloric acid, and recrystallizing two or three times from dilute alcohol. The yield in the first experiment was 24 per cent of the theoretical. Later experiments apparently yielded better results, but the resulting quantity of acid was not weighed. The pure nitro acid

melts at 182° to 183°. It is of a light yellow color and has no taste. It is slightly soluble in hot water, from which, on cooling, it crystallizes in radial tufts of needles. It is insoluble in petroleum ether and sparingly soluble in ordinary ether. It is very soluble in warm alcohol and glacial acetic acid. The acid was analyzed by determining the amount of silver in the silver salt, which yielded results as given below. In the second analysis the silver salt had darkened somewhat by being allowed to remain in contact with a solution of silver nitrate during the making.

	Calculated.	Found.	
		I.	II.
Ag	30.09	30.09	30.59

The alkali salts of this acid yield deep yellow solutions. The neutral solution of the ammonium salt yields precipitates with the following: Cupric sulphate, aluminic chloride, lead nitrate, manganous chloride, cobaltous chloride, magnesium sulphate, ferric chloride, ferrous sulphate, cadmium chloride.

The cadmium salt is white and crystalline. Nickel, calcium, strontium, and barium salts yielded no precipitates with the dilute solutions used. The silver salt, made by precipitating the neutral ammonium salt with silver nitrate, separates in light pink, curdy lumps. It is sufficiently soluble in water to yield a slight turbidity when hydrochloric acid is added. Its solubility was determined to be 1 part in 2180 parts of water at ordinary room temperature. When pure and dry it is very stable, and suffers no apparent change even when exposed to direct sunlight for several hours. It melts with decomposition at about 220°.

The barium salt is prepared by adding a slight excess of barium hydroxide to a strong solution of the ammonium salt, boiling off the ammonium, precipitating the excess of barium with carbon dioxide, and filtering hot. On cooling, the barium salt separates out in flesh-pink, pearly scales. One part of the salt dissolves in 122 parts of boiling water and in 948 parts of cold water. Before making an analysis it was dried over sulphuric acid for several days, and then heated in the air-bath for three or four hours at 100° to 110°. At about

100° it took on a much deeper hue, which seemed to be permanent.

	Calculated for $Ba(R)_2 + 1\frac{1}{2}H_2O$.	Found. I.	II.
Ba	19.37	19.32	19.03
H_2O	7.64	7.35	7.06

2-Amido-4'-methyl Phenylether, $NH_2C_6H_4OC_6H_4CH_3$.—The amido ether was prepared by dissolving the previously described nitro ether in alcohol and reducing with tin and hydrochloric acid while warming on the water-bath to 40° to 50°. During the reduction the solution was sky-blue, but when the reduction was complete it usually became pink. The end of the reaction was determined by taking out a test portion, and diluting with several times its own volume of water. When this failed to cause a turbidity due to the original substance the reduction was discontinued. The tin was precipitated with hydrogen sulphide, and, on concentration on the water-bath, the hydrochloride crystallized out in short, white needles, which are very stable when dry but unstable in contact with water. The salt melts at 220°. It is much less soluble in cold than in hot water. An attempt to prepare the free base was unsuccessful. It was precipitated from the hydrochloric solution, but decomposed in a bell-jar over sulphuric acid before it could be thoroughly dried.

The chloroplatinate was prepared by the usual method. It is of a greenish-yellow color, and melts with decomposition at 150°. The salt was dried over sulphuric acid for several days and then at 100° to 110° for three hours. The loss in weight corresponded to 1.5 molecules of water. On being heated it acquires a much darker tint, but on coming in contact with the air the surface immediately assumes its original color. It is very hygroscopic and gains weight rapidly while being weighed.

	Calculated.	Found.
Pt	24.1	24.1
H_2O	3.23	3.03

Other work in similar lines is in contemplation to extend our present knowledge of the derivatives of phenyl ether.

LABORATORY OF ORGANIC CHEMISTRY,
UNIV. OF WISCONSIN,
June 30, 1900.

NOTE.

Notes on Industrial Chemistry.[1]

It is difficult for the chemist who is not in direct touch with the industrial side of chemistry to keep informed of its rapid strides, and as the International Congresses held at Paris during the past summer presented a chance for the discussion of such matters the journals reporting the proceedings of these meetings contain much material that can with profit be brought to the attention of all who are interested in chemistry. In this paper a few of these matters will be briefly discussed.

The electrolytic method for the preparation of the hydroxides of potassium and sodium can be carried out with greater success in the case of the former. While a number of factories prepare potassium hydroxide by the decomposition of a solution of potassium chloride, the chlorine being used to make bleaching-powder, only one factory in Germany decomposes sodium chloride by this method. The products are separated by means of a porous diaphragm or by the use of mercury, according to the Castner process as modified by Solvay.

The Solvay process, as used for the preparation of sodium carbonate, cannot be used for the preparation of the corresponding potassium compound. This is made by passing carbon dioxide through a mixture of magnesia and a saturated solution of potassium chloride, when a difficultly soluble magnesium potassium carbonate is precipitated. Part of the carbon dioxide is driven out by heat, and the potassium carbonate extracted by water.

Carbon dioxide and sulphur dioxide, which are prepared by burning coke and sulphur or sulphides, respectively, are purified by passing them into saturated solutions of their neutral salts and decomposing the acid salts thus formed with the evolution of the pure gases. The solutions of the neutral salts can be used repeatedly for this purpose.

The method of preparing potassium ferrocyanide by heating animal matter, potassium carbonate and iron, is still used in three German and some English and American works; but in Europe the main source of supply is coal gas. When a mixture of calcium hydroxide and iron sulphate is used to remove the hydrogen sulphide, the following reactions take place:

[1] Based on articles contained in the Revue générale de Chimie, Tome III, Nos. 1, 2, and 4; and in Die chemische Industrie for Oct. 1, 1900.

$$FeSO_4 + Ca(OH)_2 = CaSO_4 + Fe(OH)_2;$$
$$2Fe(OH)_2 + O + H_2O = Fe_2(OH)_6;$$
$$Fe_2(OH)_6 + 3H_2S = Fe_2S_3 + 6H_2O;$$
$$Fe_2S_3 + 3O + 3H_2O = Fe_2(OH)_6 + 3S.$$

During this process the cyanogen compounds are also absorbed by the ferric hydroxide, and, after the mass has become useless on account of the accumulation of sulphur, they are isolated in the following manner: The mass, which contains sulphide of iron, sulphur, salts of lime, and free lime, ferrocyanides and sulphocyanides of iron and ammonia, is treated with water to remove the soluble part, and the residue, after treatment with lime to form the calcium ferrocyanide, is converted, by the action of potassium chloride, into an insoluble compound of potassium and calcium ferrocyanide, which is decomposed by potassium carbonate with the formation of potassium ferrocyanide. In one English factory the gas is passed through a solution of an iron salt and the Prussian blue formed is decomposed by caustic potash. In one factory in Germany the "vinasses" are heated and the cyanogen compounds formed from the decomposition of the trimethylamine are converted into Prussian blue as in the other method. In 1899 the production of potassium ferrocyanide in France, Germany, England, Belgium, Holland, and the United States amounted to 10,500 tons, of which 50 per cent was converted into potassium cyanide. Potassium sulphocyanide, which is also obtained from the coal gas, is only made in comparatively small quantity and is converted into a copper salt which is used as a constituent of paint for the hulls of vessels. This copper salt acts as a poison to the crustacea which foul the hulls of ships.

Potassium cyanide, which, since its introduction as a solvent for gold in extracting the latter from its ores, has been manufactured in such quantities, is prepared almost entirely by one method, that of Rössler and Hasslacher. After the introduction of the electrolytic method for the preparation of aluminium some disposition had to be made of the metallic sodium which had formerly been used in the preparation of this substance. This problem was solved by the introduction of a method for the preparation of potassium cyanide which was very simple and in which the sodium could be used. By its action on potassium ferrocyanide a mixture of potassium and sodium cyanides and iron is formed. By washing and crystallizing the product, the substance which is sold under the name of potassium cyanide is obtained in such amounts and so cheaply that it has displaced the synthetical method

and the Liebig method, in which the ferrocyanide was treated with potassium carbonate. Attempts have been made to utilize the nitrogen of ammonia for the preparation of potassium cyanide; but the processes devised for this purpose are as yet in the experimental stage. About 7500 tons of the cyanides, which are used in the extraction of gold and in plating on account of the formation of soluble double cyanides, were manufactured in 1899.

The amount of sulphuric acid manufactured has been steadily increasing from year to year at a rapid rate. The following figures of the industry in Germany will perhaps give a clearer idea of the change: In 1882 the amount was 358,149 tons; in 1890 the amount was 627,392 tons; and in 1897 the amount was 845,582 tons.

As yet very few of the mechanical ovens for roasting the ores have been introduced into Germany, although they are used in a number of factories in the United States. The ovens used in Europe are of the Perret-Malétra type, with 4, 5, or 6 plates, in which the source of heat is the burning sulphur, if fine ore is to be used; but in some places a series of muffles are placed one above another and the zinc blend is roasted by the passage of the hot gases over the muffles in succession, while the ore is removed from a higher to a lower muffle as the process proceeds.

There has been practically no change in the Glover tower except the introduction, in several cases, of two small towers in place of one large one.

Each manufacturer or builder has his own peculiar idea regarding the size and shape of the leaden chambers, some using several large ones, and others a number of small ones. The usual size of the chambers in Europe is 8×12 meters, while in America the manufacturers often use 12 chambers 8 meters long. The general shape is that of a parallelopiped; but many devices have been introduced to bring about a more thorough contact of the gases and so produce a better yield. In some cases the entrance tubes for the gases are arranged tangent to the sides and the exit tubes at the centre, so that a circular motion is imparted to the gases. In others, one chamber is placed inside of another, thus economizing space and overcoming, to some extent, the fluctuations in temperature. In some furnaces devices are used to cool the interior of the chamber, such, for instance, as columns or pipes, through which cool air can circulate, extending up into the chambers. The Glover tower has greatly increased the efficiency of the process, as 15 to 20 per cent of the acid is formed

there. The amount of nitrate used to produce 100 kilograms of the monohydrate varies from 0.83 to 1.12 kilograms.

In the concentration of the acid in platinum vessels great care has to be observed to prevent a loss of platinum, as a small amount of impurity may produce a marked effect. The purer the acid the less the danger of attack; but with acids of equal purity the amount of loss increases with the concentration. For many years acid of 93 per cent monohydrate was considered satisfactory for all purposes; but the demand for an acid of 95 to 98 per cent has necessitated the introduction of other material than platinum for concentration vessels, and one of platinum and gold is now used with success for this purpose. A vessel which can furnish 10,000 to 12,000 kilograms in twenty-four hours would have to contain 56 kilograms of pure platinum; but one containing 52 kilograms of platinum and 4 kilograms of gold can be used to greater advantage as the loss in the case of the platinum vessel is 1 gram per ton and, at the highest, 0.1 gram per ton for the platinum and gold vessel, a saving of about $940 a year. The latter also requires less heat and less attention. An alloy of platinum and cast-iron and simple cast-iron vessels have been used with some success if the material is carefully selected. Of course the deterioration is more rapid, but the loss is proportionately no greater and the amount invested is much less. Porcelain vessels have also been used for this purpose.

Within a few years a method for the preparation of sulphuric acid has been introduced which does not necessitate the use of the cumbersome leaden chamber, but is based on an observation made by Berzelius of the influence of one substance in producing a reaction between two other substances without itself undergoing any change. He called this catalytic action, and substances of this nature were called contact substances. A number of observers have repeated the experiments made in 1831 by Philipps, when sulphurous acid and oxygen were made to combine by the presence of a third substance, and have attempted to apply this method for the preparation of sulphuric acid on the large scale. Winkler and others have used platinum asbestos as the contact substance; but all efforts in this direction for several years led to the conclusion that the method was only applicable to the preparation of the fuming sulphuric acid with a maximum yield of 67 per cent.

In 1898 an observation was made which rendered it possible to use this method with great success, and at the present time at least eight firms are preparing the acid by this method. It was found necessary to reduce the temperature of

the gases in order that they might combine, and this can be done in a number of ways. This method has probably not been generally adopted on account of the lack of knowledge of the details, and a belief that it is as yet only in the experimental stage. The royalty is also high and owners of the old style plants cannot afford to cast these aside at once, and in fact there is still doubt as to the part this improvement will play in the industry.

As yet the price has not been affected; but when the supply from this source increases, the chamber acid methods may have to be improved to enable them to compete with the new method, and the matter may adjust itself by a division of labor, when the concentrated acid will be made by the contact method and the dilute acid by the chamber process at a profit to the manufacturers of each.

The advantages claimed for the new method are a large economy in space, a reduction in capital invested of 30 to 40 per cent, and the saving of all expense for nitrate or concentration. The price of this acid is, however, 15 per cent less.

J. ELLIOTT GILPIN.

REVIEWS.

LEÇONS DE CHIMIE PHYSIQUE, PROFESSÉES A L'UNIVERSITÉ DE BERLIN. PAR J. H. VAN'T HOFF, Membre de L'Académie des Sciences de Berlin, Professeur Ordinaire a L'Université et Directeur de L'Institut de Physique de Charlottenbourg. Ouvrage traduit de L'Allemand par M. CORVISY, Professeur agrégé au Lycée de Saint-Omer. Troisième Partie. Relations entre les Propriétés et la Composition. Avec un Portrait gravé de l'auteur. Librairie Scientifique, A. HERMANN. Paris, 1900. pp. 153.

The appearance in French of the first and second parts of Van't Hoff's "Vorlesungen" has already been noted in this Journal (Vol. 23, 531). It only remains to call attention to the completion of the French edition. The strong desire on the part of chemists in France to keep in touch with the latest developments in physical chemistry, is shown by the short time which has elapsed between the appearance of the German original and the French translation of this brilliant work. The earlier parts appeared in French some time before the German edition was completed, and the third and last part has been published in French in less than a year after it appeared in the original.

As will be seen from the title-page this part of the book deals with relations between properties and composition. Two classes of properties are taken up—physical and chemical—and the effect of composition upon each pointed out.

The book as a whole, including dynamics, statics, and relations between properties and composition, can scarcely be regarded as a systematic and comprehensive text-book on physical chemistry, nor does it pretend to be such. We find, however, in this work all of the most important phases of physical chemistry discussed, and like all work from the pen of Van't Hoff, it contains new and suggestive ideas at every turn. This book is not adapted to the beginner in physical chemistry, but is essential to every one who would teach this branch of science. H. C. J.

EINFÜHRUNG IN DIE STÖCHIOMETRIE, ODER DIE LEHRE VON DER QUANTITATIVEN ZUSAMMENSETZUNG DER KÖRPER UND IHREN MIT DIESER ZUSAMMENHÄNGENDEN EIGENSCHAFTEN, MIT RECHENBEISPIELEN. Für Studierende und Chemiker. Von DR. JOACHIM BIEHRINGER, a. o. Professor au der Herzoglichen Technischen Hochschule zu Braunschweig. Mit 18 Abbildungen und einer Tafel. Friedrich Vieweg und Sohn. Braunschweig, 1900. pp. 498.

Many of the works on stoichiometry which have hitherto appeared have been rather dry reading. This is partly due to the nature of the subject. Most of the relations which have been established in this field are purely empirical, and we have little or no conception as to what they mean. It is not a simple matter to present such relations in an attractive and interesting form. The book in hand, however, is interesting reading. The author has shown how it is possible to deal in an attractive manner, with facts which are not correlated by any wide-reaching generalization. This has been accomplished, in the first place, by a complete mastery of the material in hand, and, in the second place, by an unusually clear and lucid method of presentation.

This book covers rather more ground than the average work which deals with the subject of stoichiometry even in a broad way. This will be seen from the contents. Division one, on the laws of combination and the atomic theory, includes the laws of definite and multiple proportions and the historical development of the atomic theory. The second and third parts of the book deal with gases and liquids, and especially the physical properties of matter in these states of aggregation.

Division four is devoted to solutions, and here some of the fundamental conceptions of the newer physical chemistry are introduced. A large part of this division of the book is given up to the theory of dilute solutions, including a discussion of osmotic pressure, lowering of freezing-point, rise in boiling-point, methods of determining molecular weights in solution, etc.

The physical properties of solids are taken up after solu-

tions, and division six is devoted to relations between the atomic weights, including, of course, the Periodic System.

There might be some question as to the best order of arrangement of the material in the latter part of the book. As we have now so many well-defined examples of solid solutions, would it not be better to study gases, liquids, and solids, and then take up solutions in a broad way, including mixtures of matter in every state of aggregation with matter in the same and every other state ?

Again, ought not any relations between the atomic weights be pointed out directly after the methods for determining atomic weights have been considered ?

These questions are not raised with any desire to criticize the book. It is obvious that these are minor points and in no wise detract from its great merit. It is not too much to say that Biehringer has given us one of the most readable and valuable books on stoichiometry which has ever been published.

H. C. J.